The Use of Ionising Radiation in the Treatment of Thyrotoxicosis

A Radiobiological, Clinical and Socio-Medical Study

by

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A B S T R A C T O F T H E S I S

The investigations reported in this thesis arose from an interest in the problems inherent in the treatment of thyrotoxicosis with radioiodine (^{131}I). This form of therapy combines the short term disadvantage of slow and unpredictable control of symptoms and the long term disadvantage of unpredictable late onset hypothyroidism. In a group of thyrotoxic patients, increasing the mean therapy dose of ^{131}I hastens the rate of control but raises the incidence of hypothyroidism: smaller doses diminish the hypothyroid rate but lengthen the time for control of symptoms.

In view of these difficulties it was decided to pursue three lines of investigation. Firstly, a series of radiation studies were carried out on the rat thyroid to obtain more information about relevant radiobiological mechanisms. Secondly, therapeutic trials of low doses of ^{131}I and γ-radiation (Cobalt-60) were conducted in an attempt to reduce the incidence of late onset hypothyroidism. Thirdly, a method of automatic life-long follow-up was initiated to deal with the problem of undiagnosed hypothyroidism occurring in patients treated in the past with ^{131}I. In addition two other subsidiary studies emerged in the course of the work. The experimental model used in the radiation studies consisted of the "goitrogenic response" of the rat thyroid to methylthiouracil administration. As a prerequisite to these investigations it was necessary to define the normal goitrogenic response in relation to both/

both gland weight and thyroid follicular cell population. This, in turn, involved the use of certain quantitative histological techniques. As some of these were found to be unsatisfactory a study of their theoretical foundations was undertaken with a view to defining their practical usefulness and limitations. The results of all these investigations are presented in the various sections of this thesis and the main conclusions are summarised below.

Section I:

The goitrogenic response of the rat thyroid to methylthiouracil is triphasic, consisting of a lag phase of 2 days and a logarithmic growth phase lasting 10 days followed by a plateau phase. As the mean follicular cell concentration remains constant throughout all three phases, the changes in the total follicular cell population with methylthiouracil administration parallel those for gland weight. Finally, contrary to expectations, follicular cell hypertrophy does not precede hyperplasia in the goitrogenic response but is concomitant with it.

Section II:

The goitrogenic response described above was used as the model for studying the effects of x-radiation in the range 0 to 2,500 rads on the rat thyroid. It was again found that the follicular cell concentration remained constant irrespective of dose of x-rays, time after/

after irradiation and time on methylthiouracil. Consequently, as in the normal goitrogenic response, the changes in thyroid weight can be equated with the changes in the total thyroid follicular cell population.

In the dose range employed there was no evidence of immediate or delayed cell death although 1,000 rads x-rays left fewer than 1% of the follicular cells reproductively intact. The corollary of this is that doses of x-rays which do cause significant cell death must leave only an infinitely small number of cells reproductively intact. It is therefore concluded that a "radiation partial thyroidectomy", which requires the combination of significant cell death and significant cell reproductive survival to ensure continuance of an adequate functioning thyroid cell population, is intrinsically impossible using homogeneous thyroid irradiation.

Section III:

This section reports the effects of doses of x-rays in the range 0 to 2,500 rads on rat thyroid function as measured by the iodide trapping capacity of the gland. The absence of any significant effect up to 12 months after 1,000 rads x-irradiation is consistent with the absence of significant cell death reported in Section II and indicates that there is no significant production of viable, non-functioning, follicular cell mutants with doses of this order.

The/

The fluctuations in iodide trapping during the first 96 hours after 500 to 2,500 rads x-radiation suggests that short term damage of a reversible nature has occurred. This may be due to cytoplasmic (enzyme) damage or to arterial spasm resulting in temporary tissue ischaemia.

Section IV:

In this section the results of the clinical trials of low doses of ^{131}I and γ-radiation (^{60}Co) in the treatment of thyrotoxicosis are reported. Of 30 patients treated with doses of ^{131}I calculated to deliver 2,400 rads to the thyroid only 11 were euthyroid 2 to 3 years later. Persisting hyperthyroidism, relapse or hypothyroidism occurred in the remainder. In the 28 patients treated by γ-radiation 25 relapsed after antithyroid drugs were withdrawn. It is concluded that there are no indications for the treatment of thyrotoxicosis with external radiation alone and that a "predictable radiation partial thyroidectomy" with ^{131}I is impossible in the individual patient. The possible radiobiological mechanisms underlying these observations are discussed in relation to these conclusions.

As a therapeutic compromise, a treatment policy which attempts to postpone the development of thyroid failure to a time beyond the patient's life expectancy is presented.

Section V:/

Section V:

In recognition of the high cumulative incidence of hypothyroidism following ^{131}I therapy and the unreliability of patients in long term self administration of thyroxine replacement therapy, an automated life-long follow-up scheme has been put into operation in the Aberdeen area. This is carried out by the patients' family doctor with the aid of a serum PBI estimation and a computer derived diagnostic index for hypothyroidism. This has proved efficient in the detection of hypothyroidism and extremely economical of medical manpower with an 89% reduction in the demands on the hospital out-patient clinic. A one year pilot survey, in the Manchester area, of a group of patients treated with ^{131}I in 1957-58 has demonstrated both the need for such a scheme and the difficulties inherent in its retrospective application.

Section VI:

In this section a set of propositions in statistical geometry are considered in relation to certain quantitative histological techniques concerned with the estimation of numbers, volumes and surface areas of tissue structures. Proofs of these propositions are presented and the practical limitations of the various techniques discussed.

A C K N O W L E D G E M E N T S

The investigations reported in this thesis were carried out by me in the Department of Therapeutics and Pharmacology, Aberdeen University between 1964 and 1967. However, a group of studies related to one another by their bearing on different aspects of a clinical problem, inevitably involves the co-operation of colleagues and I am pleased to acknowledge their help.

I would first like to express my gratitude to Professor A.G. Macgregor and Dr. J. Crooks who created the environment in which this work could take place. I would like to thank Dr. M.T. Harrison who co-operated in the joint clinical trials of low doses of radioiodine and external radiation therapy (^{60}Co) carried out in Aberdeen and Glasgow and Professor Sir Edward Wayne and Dr. W.D. Alexander who gave permission to study their patients at the Thyroid Clinic, Glasgow Western Infirmary. Dr. T.J. Buchanan has been of invaluable assistance with the mathematical aspects of the radiobiological and statistical geometric studies and I have also benefited from discussions with Mr. E.J. Harding and Dr. W.Z. Billewicz in this connection. Mr. J.A.R. McIntosh assisted with the animal radiation procedures and Dr. E.F. Ridley supervised the γ-irradiation of the thyroid in the external radiation therapy trial. Dr. E.C. Easson, Director of the Christie Hospital and Holt Radium Institute, Manchester, gave permission for the study of the group of patients there and I am extremely grateful to Dr. K.E. Halnan/

Halnan and Dr. M.B. Duthie for their help in overcoming the considerable logistic difficulties in this investigation.

I have also had the benefit of technical assistance from Mr. D.N. Noble and Mr. W.R. Ferrier. Mr. D.P. Hammersley illustrated the irradiation exposure box, Mr. W. Topp was responsible for the photography and the thesis was typed by Mrs. M. Brand. I would like to thank all of these for their skill and patience.

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C O N T E N T S

A P O L O G I A

Since the high incidence of iatrogenic hypothyroidism (40% eight years after treatment) in thyrotoxic patients treated with radioiodine was appreciated, the radiobiology of the thyroid gland has become a subject of great clinical importance. The method of treatment in its orthodox form is clearly unsatisfactory and attempts are being made to improve it in various centres. Unfortunately these efforts are taking place against a background of inadequate radiobiological information.

External radiation was once used extensively in the treatment of thyrotoxicosis and many reports exist claiming that the disease could be cured by this means. Hayes (1927) claimed that 62% of his cases were cured and that 14% improved. Groover, Christie, Merritt, Coe and McPeak (1929) claimed an 89% cure rate and 9% improved. Hayes (1927) warned of the possibility of inducing hypothyroidism by radiation overdosage but gives no indication of the incidence of this occurrence. Unfortunately it is impossible in many cases to ascertain what rad dose the patients received as complete physical details of the x-ray machine and procedure are not supplied. Furthermore, in all cases the total doses were fractionated over several weeks and calculation of the one dose equivalent is impossible. It is estimated by Goolden (1964) that the total cumulative dose could not have exceeded 3,500 roentgens. It is therefore of interest that 5 cases of hypothyroidism were found/

found by Markson & Flatman (1965) within three years of x-ray therapy for laryngeal cancer and that Einhorn and Wikholm (1967) found a diminished response to T.S.H. in 41 similar patients 10 years after therapy with overt hypothyroidism in 3 cases.

Radioiodine (^{131}I) when it was introduced by Hertz in 1941 offered a golden therapeutic opportunity. Here was an agent which could seek out its own target and then destroy it, the degree of destruction, it was thought, being proportional to the millicurie dose administered. Numerous trials of this agent have been reported. In the series reported by Greig, Crooks and Macgregor (1966), 38% remained thyrotoxic and 25% became hypothyroid with one dose. Of those requiring a second dose (38%) 37% remained thyrotoxic, 29% became euthyroid and 27% became hypothyroid. Occasionally three or four doses were required. The rate of control of hyperthyroidism with radioiodine therapy is therefore slow and the incidence of hypothyroidism is very high and increases with the number of doses given and the length of time after treatment. The same pattern has been reported by Beling & Einhorn (1961), Dunn & Chapman (1964), Green & Wilson (1964), McGirr, Thompson & Murray (1964) and Einhorn & Wicklund (1966). The situation was summarised by Crooks (1965). In his series of patients 40% remained thyrotoxic 8 months after therapy and 40% were hypothyroid 8 years after therapy with no evidence of a plateau having been reached/

reached in the cumulative incidence. A trial of a low dose regime of ^{131}I has been reported by Smith & Wilson (1967). They gave "calculated rad doses" of "3,500 rads" in contrast to orthodox levels of "7,000 to 10,000 rads". This procedure lowered the incidence of hypothyroidism at 5 years after therapy from 29% to 7.4% but it remains to be seen if a stable plateau will be reached in the cumulative incidence of hypothyroidism. Furthermore, as a result of the lower dose employed there was an even greater delay in the attainment of the euthyroid state and antithyroid drugs were required in a higher proportion (64%) of patients compared to 43% in the conventional dose group.

These clinical observations point to the basic conflict in radioiodine therapy, i.e. that high doses effect rapid control but cause a high hypothyroid rate; lowering the dose decreases the hypothyroid rate but lengthens the period for control of symptoms unless antithyroid drugs are used. In the case of deliberately induced mild hypothyroidism in cases of angina pectoris the conflict disappears as this result can almost be guaranteed: Blumgart, Freedburg & Buka (1948), Blumgart, Freedburg & Kurland (1955). In general, however, the ablative dose required is huge (e.g. 50 m.Ci) compared to that used in the conventional treatment of thyrotoxicosis (6-12 m.Ci). This may be due to greater radiosensitivity of the cells of the thyrotoxic gland or to their increased/

increased uptake of the administered dose. On the other hand as yet unknown biological phenomena may be operating to produce this disparity in apparent radiosensitivity.

These clinical effects are in line with studies of the function and histology of the radioiodine treated human thyroid gland. The functional changes have been described by Dobyns, Vickery, Maloof & Chapman (1953) and Eckert, Green, Kilpatrick & Wilson (1960). In patients rendered euthyroid the absolute uptake of a tracer dose of radioiodine is within the normal range although the residual thyroid tissue remains overactive as evidenced by a high turnover rate of the tracer. The response to TSH is reduced and there is little suppression of thyroid function by triiodothyronine. That the functional reserve is reduced is demonstrated by the ease with which the serum P.B.I. can be lowered by antithyroid drug administration. These findings are in contrast to the behaviour of the remnant after partial thyroidectomy which after a period of five years often comes to resemble the normal thyroid both functionally and histologically. The histological picture in the radioiodine treated cases is compatible with the functional observations and has been described by Dobyns et al. (1953), Freedburg, Kuland & Blumgart (1952), Lindsay, Dailey & Jones (1954) and Curran, Eckert & Wilson (1958). The acute changes consist of nuclear pyknosis, cell death, small vessel thrombosis and oedema of the stroma. In the long term, there are small islands of functioning/

functioning cells embedded in large volumes of fibrous tissue. The follicles are small and irregular with little colloid storage; the follicular cells are large and many of them have bizarre nuclear forms.

In none of these series were malignant cells observed in the histological sections. In adults no significant incidence of thyroid cancer has been found either after external x-ray therapy (Quimby & Werner, 1949), or after radioiodine therapy (Hollingworth, Hamilton, Tamagaki & Beebe, 1963). In children and adolescents, however, a definite association between external x-irradiation of the neck and the subsequent development of thyroid cancer has been established (Goolden, 1964). It has also been suggested by Sheline, Lindsay and Bell (1959) that thyroid cancer may follow the use of radioiodine for treating hyperthyroidism in childhood. In Doniach's animal experiments (1953) a significant number of rats developed thyroid carcinoma after being treated with low doses (5 to 30 μCi) of ^{131}I followed by methylthiouracil. No such tumours were found in a group of rats given 100 μCi ^{131}I. One possible interpretation of this phenomenon is that all doses of radiation cause cancerous mutations but with really high doses the cancer cells are rendered incapable of division. It is unfortunate for this reassuring hypothesis, however, that Goldberg & Chaikoff (1952) described thyroid cancer in rats within two years of their receiving/

receiving 400 μCi of ^{131}I.

Another possible biological hazard of radioiodine therapy is leukaemogenesis but no significant incidence of this has been observed: Pochin (1960), Green, Fisher, Miller & Wilson (1961) and Werner, Gittelshon & Brill (1961). Similarly no significant incidence of congenital malformations has been found in the children of mothers treated with radioiodine: Means, DeGroot & Stanbury (1963).

The aim of radioiodine therapy is to achieve a "radiation partial thyroidectomy", which necessitates significant cell death combined with significant cell survival, but it remains to be seen if this is possible within a reasonable period of time. Clinical trials, so far, have not been conclusive and it is more likely that the solution will come from purer radiobiological situations in which fundamental changes in cell behaviour following irradiation can be assessed in a quantitative manner.

In recognition of the problems presented in the above review it was decided to initiate a group of relevant radiobiological, clinical and socio-medical investigations in an effort to understand better and improve the treatment of thyrotoxicosis with ionising radiation.

Firstly in order to obtain more information about the radiobiological problems of whole organ irradiation it was decided to investigate the effects of accurately measured graded doses of x-rays/

x-rays on the goitrogenic response of the rat thyroid to methylthiouracil administration. As a prerequisite to this, however, it was necessary to define the normal goitrogenic response more precisely and by reccurse to quantitative histological techniques to convert the goitrogenic response for thyroid weight into a growth curve for the thyroid follicular cell population. This enabled the effects of x-radiation on the growing thyroid follicular cell population to be expressed in terms of cell population kinetics and the results compared with those obtained in radiobiological studies of cell populations in tissue culture. The results of these investigations are presented in Sections I and II of this thesis. In addition, the effects of x-radiation on thyroid function were studied with particular regard to determining the possible contribution of non-functioning follicular cell mutants to any decrease in thyroid function (Section III).

Secondly, in the light of the results of the trial of low doses ^{131}I in the treatment of thyrotoxicosis reported by Smith & Wilson (1967) it was decided to conduct a trial of even smaller doses of ^{131}I in an effort to further diminish the incidence of hypothyroidism. In addition, because of the trend to lower doses of ^{131}I the possibility of using external radiation therapy suggested itself, particularly in view of the reports of successful x-ray therapy discussed above. With modern radiotherapy techniques (e.g. ^{60}Co derived/

derived γ-radiation) it was hoped to improve on the results obtained in the pre-^{131}I era and to overcome the problems of dosimetry inherent in ^{131}I therapy. Because of the relatively small number of new thyrotoxic patients presenting annually in Aberdeen these trials were carried out in two centres. The trial of low doses of ^{131}I was conducted at the Thyroid Clinic, Glasgow Western Infirmary with the co-operation of Dr. M.T. Harrison. The trial of external radiation therapy was carried out at the Thyroid Clinic, Aberdeen Royal Infirmary (Section IV).

Thirdly, it was clearly necessary to find a solution to the problem of undiagnosed hypothyroidism in those patients who had been treated with ^{131}I in the past. Consequently an automated method of life-long follow-up was designed and put into operation in the Aberdeen area (North-East Scotland Regional Board of Management) with the aim of detecting hypothyroidism in patients who were previously undiagnosed and in those who had established hypothyroidism but who had failed to adhere to their thyroxine replacement therapy regime. As the Aberdeen area is particularly favourable to the success of such a scheme it was decided to test its efficacy under the most adverse conditions. For this purpose a group of patients treated at the Christie Hospital, Manchester in 1957-58 were chosen. These patients not only resided in a metropolitan area but in the majority of cases had not been reviewed for/

for eight years. They therefore provided an opportunity to assess both the magnitude of the public health problem resulting from late onset hypothyroidism and the operational difficulties inherent in the follow-up scheme. (Section V).

Finally, in the course of the histological investigation of the goitrogenic response of the rat thyroid it emerged, from a review of the relevant literature, that there was considerable confusion concerning the theory and practical application of various quantitative histological techniques. Consequently a study of the theoretical basis of these methods was undertaken in an attempt to define their practical limitations and usefulness. (Section VI).

SECTION I

GROWTH CURVE OF THE RAT THYROID UNDER A GOITROGENIC STIMULUS

INTRODUCTION

Methylthiouracil administration causes the rat thyroid gland to undergo an increase in weight (the goitrogenic response): Thyssen (1947), Santler (1957), Crooks, Greig, Macgregor and McIntosh (1964). Doniach and Logothetopoulos (1955) observed that the goitrogenic response could be inhibited by ionising radiation delivered by radioiodine. Crooks et al. (1964) used the degree of inhibition of the goitrogenic response as an indicator of the effect of various levels of x-ray dosage on the thyroid cell population by comparing the growth pattern of irradiated rat thyroids with that of unirradiated controls. They found that the normal growth curve was biphasic. Furthermore, on the basis of observed differing radiosensitivities of the two phases they suggested that the shape of the growth curve might be accounted for by a predominance of cell hypertrophy in the first growth phase and a predominance of cell hyperplasia during the second. However, Thyssen (1947) reported a different form of growth curve of the rat thyroid following methylthiouracil administration consisting of a lag phase, a phase of logarithmic growth followed by a plateau phase.

In view of these conflicting reports it was decided to investigate the normal goitrogenic response of the rat thyroid gland to methylthiouracil administration and by recourse to quantitative histological/

histological techniques to see what relation existed between the increase in thyroid weight and the increase in the follicular cell population and follicular cell volume. It was hoped thereby to find a better model for investigating the effects of ionising irradiation on mammalian cells *in vivo*.

MATERIALS AND METHODS

Methylthiouracil powder was dissolved in weak sodium hydroxide (100 g. in 200 ml. N/10 NaOH) and then made up with 1% sucrose (w/v) to a final concentration of 0.1%.

Male Wistar rats weighing 220 to 280 grammes were allowed to drink at will the 0.1% solution of methylthiouracil in 1% sucrose. The sucorse encouraged the animals to drink (Crooks et al. 1964). Control animals received the 1% sucrose solution only. Fresh sucrose and methylthiouracil solutions were prepared every second day. Sample groups of test and control animals were killed before commencing methylthiouracil administration and at intervals thereafter. The precise arrangements were as follows.

Experiment P: A pilot study (P) was carried out with 14 randomly constituted groups of 5 rats each. There were 20 animals to each cage and the animals were marked distinctively according to group. Two groups (10 animals) were killed on Day 0 and one group on days 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33 and 36 after commencing methylthiouracil administration. The animals were killed by prolonged chloroform anaesthesia, their thyroid glands removed, cleaned of all muscle and connective tissue under a dissecting microscope and weighed on a torsion balance. The accuracy of the torsion balance was periodically checked over its whole range (0 to 50 mg.) against an accurate balance.

Experiment A: Nineteen groups of five rats were randomly constituted and marked distinctively according to their group. Two groups (10 animals) were killed on Day 0, eleven groups were started on methylthiouracil and the remaining six groups acted as controls. One group of the test animals was killed every second day up to 22 days and one group of the controls on days 0, 4, 8, 12, 16 and 20. The animals were killed and their thyroids removed, cleaned and weighed as in Experiment P. The glands were then fixed in neutral buffered formalin and embedded in wax in a vacuum oven. Histological sections were cut with random orientation to the longitudinal axis of the gland with a Spencer 820 precision microtome. Microtome sections of 6μ thickness were taken at intervals through the gland and stained with haematoxylin and eosin. The section with the largest surface area was chosen for the various examinations described below.

Using a Leitz Ortholux projecting microscope an image of the stained section was projected on to a screen to a net magnification of 1,000 (i.e. 1 m.m. $\equiv$ 1μ). Estimates of the number of follicular cells per unit volume were obtained by counting the number of follicular cell nuclei in each field contained within the boundaries of ten predesignated squares of a superimposed grid of known dimensions (area of one square = 543μ^2). The entire section was scanned field by field so that the final count contained samples from/

from every part of the section. In order to test the efficiency of this method of counting, every single nucleus in five non-homogeneous sections was counted and the results compared with those yielded by the sampling method repeated on three separate occasions at intervals of one week. In none of the five sections did the estimate obtained by sampling differ significantly from that obtained by counting every nucleus ($p < 0.01$).

Nuclear diameters were measured from the projected image with the aid of a pair of dividers. The mean nuclear diameter for each section was determined by measuring in one direction the diameter of 40 nuclei distributed along a straight line which was selected before projection of the image in each case. The frequency of sampling from each section (i.e. 40) was also found by trial and error to yield consistent results on repetition.

The number of follicular cells per unit volume was calculated from the following formula which incorporates Abercrombie's (1946) correction factor for section thickness.

$$N = Nc. \frac{T}{(L + T)} \frac{10^{10}}{543T}$$

$$\text{i.e. } N = \frac{Nc. 10^{10}}{543 (L + T)}$$

Where N is the number of cells per 10 m.m.^{3}, Nc is the crude mean estimate of the number of cells in one square, L is the mean nuclear diameter and T the section thickness. $\frac{T}{L + T}$ is Abercrombie's correction factor and 543T the volume of tissue under one square of the grid (μ^3).

The mean follicular cell volume (M.C.V.) was determined as follows. The intersection of the lines of the randomly superimposed counting grid (see above) formed a lattice of points which were orientated at random with respect to the projected image of the various tissue structures. The relative number of points lying over the projected image of the various tissue components such as follicular cells, colloid and stroma approximate closely to the relative volumes of these components providing the section thickness is thin relative to the various tissue components (Chalkley, 1943). This and other propositions in quantitative histology are discussed in a separate section of this thesis. One hundred points of the lattice were examined in each field and the entire section scanned field by field. The proportion of points overlying follicular cells yielded an estimate of the fraction of the total gland volume occupied by follicular cells - i.e. the relative cell volume (R.C.V.). The M.C.V. was then calculated by dividing this value by the mean number of follicular cells per 10 m.m.^{3} (N)

$$\text{i.e. } \text{M.C.V.} = \frac{\text{R.C.V. } 10^{10}}{\text{N}} \mu^3$$

Experiment G: In this experiment the animals were randomly constituted into groups of seven each. Two groups (14 animals) were killed on Day 0 and one group on days 2, 4, 6, 8, 10, 12 and 16 after commencing methylthiouracil. In all other respects the other procedures were identical to those described in Experiment A.

TABLE I: Experiment P

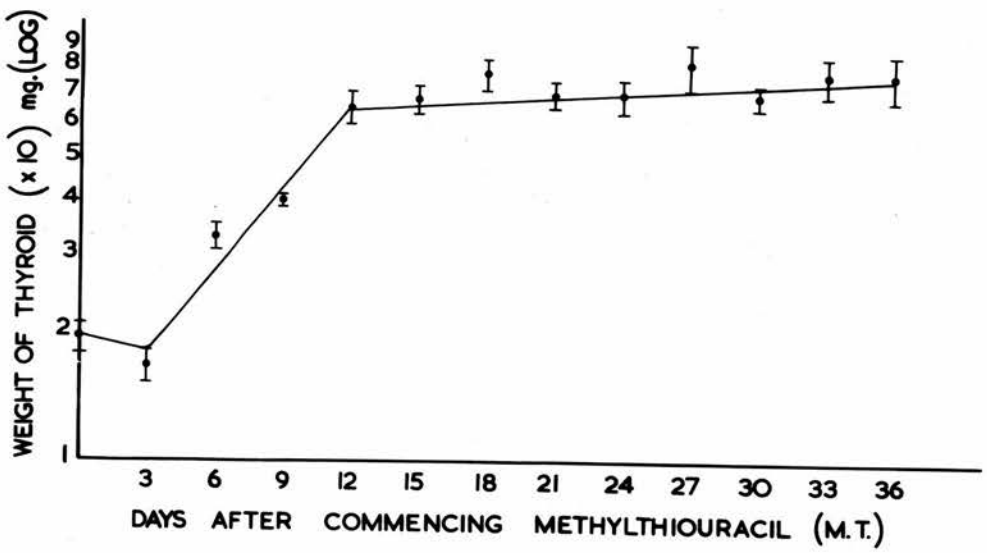
Changes in Mean Thyroid Weight with Methylthiouracil Administration

Days after commencing Methylthiouracil	0	3	6	9	12	15	18	21	24	27	30	33	36
Mean Gland Weight ± S.E. (mg.)	19.20 ±1.41	16.60 ±1.30	32.52 ±1.71	38.80 ±1.02	63.76 ±5.84	66.96 ±4.87	68.64 ±6.27	64.20 ±3.83	64.62 ±6.91	80.88 ±9.77	68.10 ±4.80	76.50 ±8.83	76.10 ±9.11

Fig. 1: Experiment P

Graphical representation of the changes in total thyroid weight with methylthiouracil administration: the log of mean gland weight ± standard errors are plotted against time in days after commencing methylthiouracil (M.T.)

Fig. 1: Experiment P



Graphical representation of the changes in total thyroid weight with methylthiouracil administration: the log of mean gland weight ± standard errors are plotted against time in days after commencing methylthiouracil (M.T.)

RESULTS

Experiment P. Table I (Appendix Table 1) shows the goitrogenic response following methylthiouracil. These results are represented graphically in Fig. 1 where the log of the mean gland weight ± the standard error is plotted against time on methylthiouracil. The lag phase, exponential phase and plateau phase described by Thyssen (1947) are clearly demonstrated.

Experiments A and G. The results of these experiments are conveniently considered together. The effects of methylthiouracil administration on mean gland weight, mean follicular cell concentration and mean follicular cell volume along with the results in untreated controls are contained in Table II (Appendix Tables 2, 3 and 4). The results of methylthiouracil administration are very similar in experiments A and G and are shown graphically in Figs. 3 and 4 respectively.

Changes in Mean Gland Weight

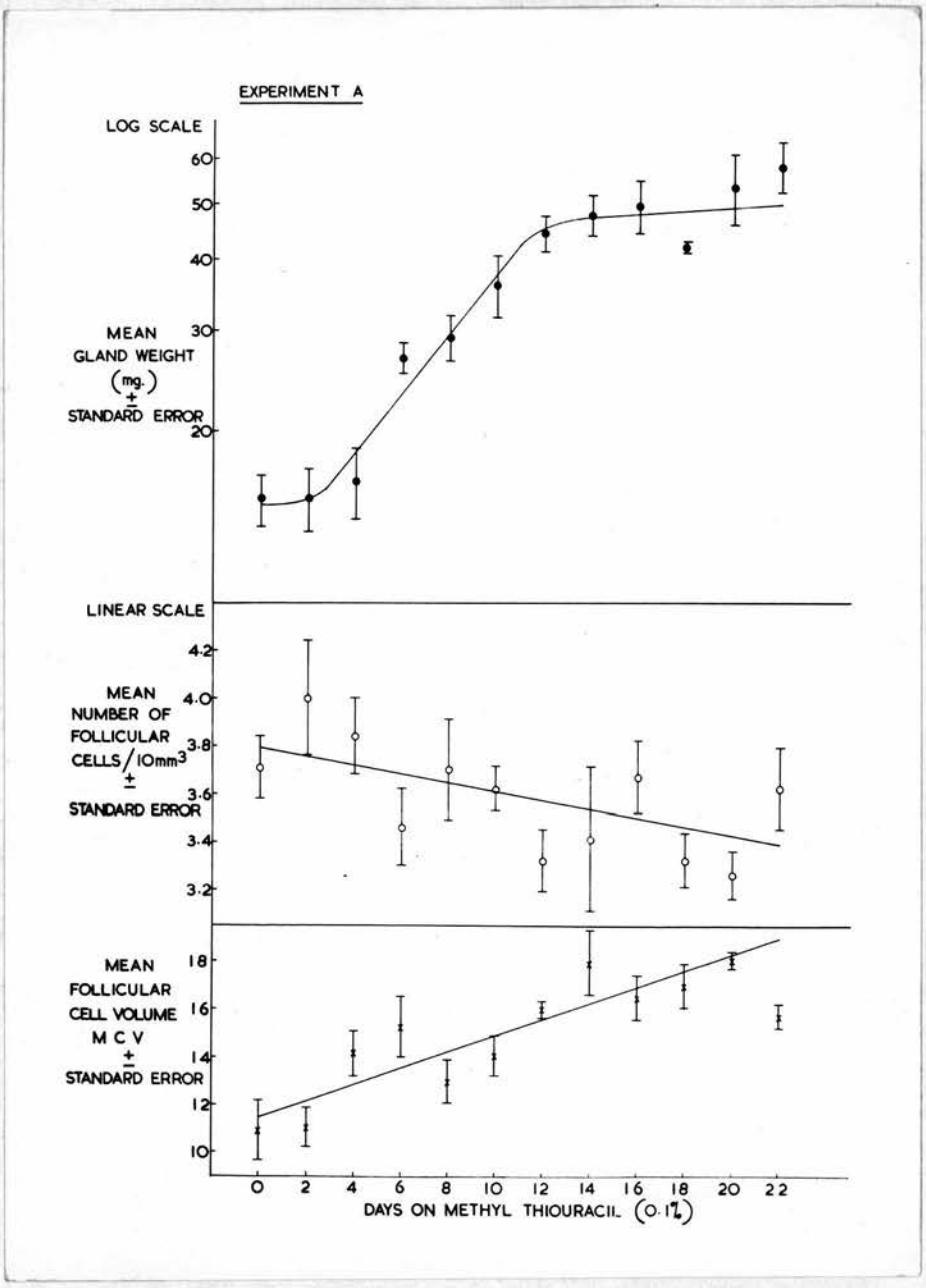
It can be seen from Figs. 3 and 4 that the growth curve for thyroid gland weight is similar to that found in Experiment P with a lag phase of two days and exponential growth for approximately 10 days until the plateau phase is reached. In the controls the gland weight remains constant with no significant regression of gland weight with time ($Y = 16.2 + 0.05X$). Analysis of variance demonstrated that this line showed no significant deviation from rectilinearity ($F < 1$) and that it had zero slope ($F < 1$).

TABLE II: Experiments A and G

Effects of Methylthiouracil Administration on the Rat Thyroid Gland Weight, Mean Follicular Cell Concentration and Mean Follicular Cell Volume

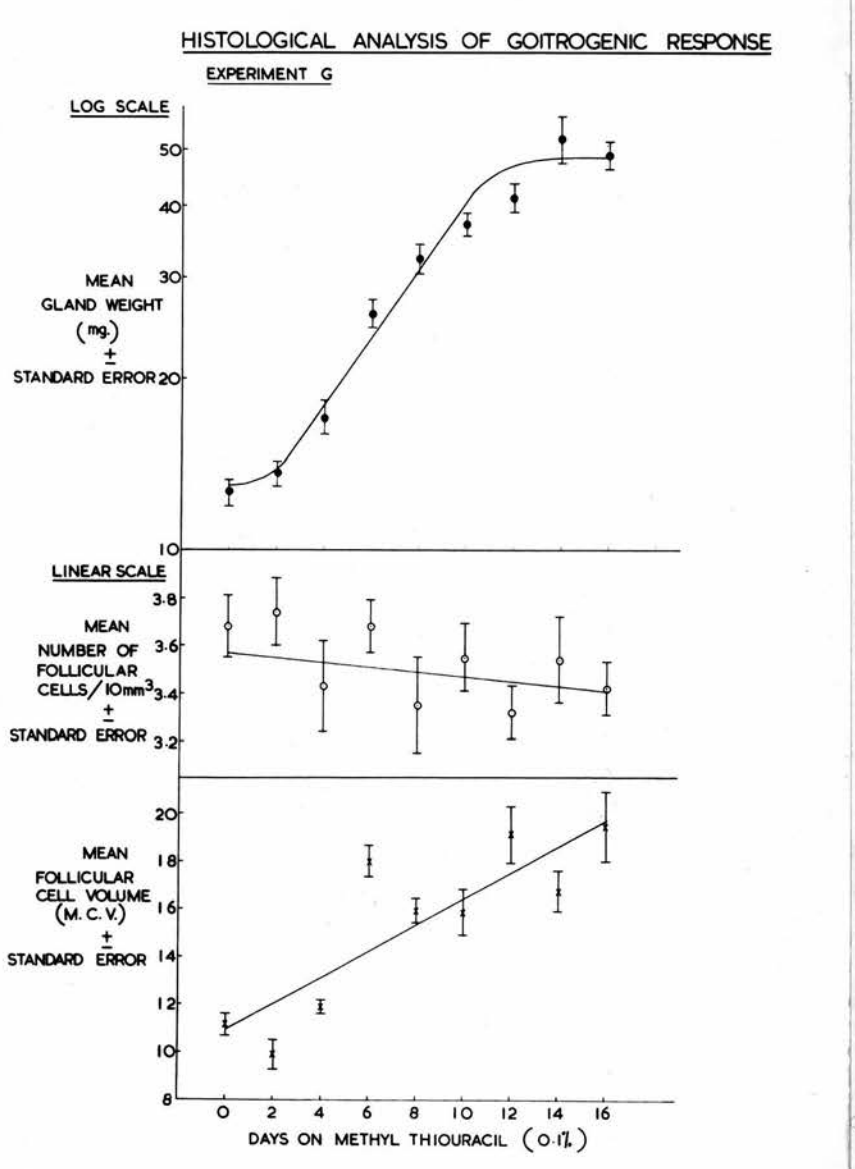
Days on Methyl-Thiouracil	CONTROLS (No Methylthiouracil			Experiment A			Experiment G		
	Mean Gland Wt. (mg.) ± S.E.	Mean Number Cells/10mm^3	Mean Cell Volume (μ^3)	Mean Gland Wt. (mg.) ± S.E.	Mean Number Cells/10mm^3	Mean Cell Volume (μ^3)	Mean Gland Wt. (mg.) ± S.E.	Mean Number Cells/10mm^3	Mean Cell Volume (μ^3)
0	17.8 ± 1.2	3.58±0.17	1135±48	15.2±1.6	3.71±0.13	1088±121	12.7±0.7	3.68±0.13	1116±42
2				15.2±1.9	4.00±0.24	1106±79	13.6±0.7	3.74±0.14	991±59
4	15.1±1.2	4.13±0.27	984±69	16.3±2.3	3.84±0.16	1413±90	17.1±1.2	3.43±0.19	1192±27
6				26.8±1.6	3.46±0.16	1520±125	25.6±1.4	3.68±0.11	1799±62
8	15.0±1.0	3.65±0.15	1070±70	29.1±2.6	3.70±0.21	1293±87	32.5±1.8	3.35±0.20	1593±50
10				36.0±4.4	3.62±0.09	1402±82	37.2±1.7	3.55±0.14	1581±94
12	17.8±1.6	3.83±0.20	1075±61	44.2±3.2	3.32±0.13	1594±33	41.2±2.0	3.32±0.11	1918±109
14				47.5±3.7	3.41±0.30	1797±133	52.5±4.9	3.54±0.18	1672±84
16	16.4±1.2	3.75±0.35	1113±132	49.1±5.2	3.67±0.15	1647±90	49.0±2.5	3.42±0.11	1943±143
18				41.8±0.5	3.32±0.11	1698±97			
20	18.0±1.1	3.66±0.11	1072±50	53.0±7.4	3.26±0.10	1802±36			
22				57.7±5.6	3.62±0.17	1571±50			
Regression Equation Y = a + bx	Y = 16.2+0.05x	Y = 3.82-0.01x	Y = 1069+0.5x		Y = 3.79-0.02x	Y = 1148+34x		Y = 3.57-0.01x	Y = 1100+54x
Analysis of Variance of Regression: F Ratio for b ≠ 0	<1	< 1	<1		1.2 (p>0.05)	46 (p<0.01)		< 1	520(p<0.01)
Analysis of Variance of Regression: F Ratio for Dev. from Rectilinearity	<1	< 1	< 1		< 1	< 1		< 1	6.9(p<0.01)

Fig. 2: Experiment A



Graphical representation of the changes in log mean thyroid gland weight, mean number of cells per unit volume (10 m.m.^{3}) and the mean cell volume (M.C.V.) plotted against days after commencing methylthiouracil (M.T.)
All ± standard error.

Fig. 3: Experiment G



Graphical representation of the changes in log mean thyroid gland weight, mean number of cells per unit volume (10 m.m.^{3}) and the mean cell volume (M.C.V.) plotted against days after commencing methylthiouracil (M.T.)
All ± standard error.

Changes in Mean Follicular Cell Concentration

In experiments A and G the mean number of follicular cells per unit volume showed no significant regression with time on methylthiouracil with $Y = 3.79 - 0.02X$ and $Y = 3.57 - 0.01X$ respectively. Both these lines showed no significant deviation from rectilinearity ($F < 1$) and both had zero slope ($F = 1.2$ and $F < 1$ respectively). In the controls there was no significant regression of mean follicular cell concentration with time ($Y = 3.82 - 0.01X$) with no significant deviation from rectilinearity ($F < 1$) and no significant deviation from zero slope ($F < 1$).

As the number of follicular cells per unit volume remains constant irrespective of time on methylthiouracil it can be concluded that the changes in gland weight parallel the changes in the total follicular cell population in the thyroid gland.

Changes in Mean Follicular Cell Volume (M.C.V.)

In both experiments A and G the M.C.V. showed an increase with time on methylthiouracil with regression equations of $Y = 1148 + 34X$ and $Y = 1100 + 54X$ respectively and significant deviation from zero slope ($p < 0.01$). In experiment A there was no significant deviation from rectilinearity ($F < 1$) although this did not hold in experiment G ($F = 6.9$ and $p < 0.01$). In the controls there was no significant change in the M.C.V. with time ($Y = 1069 + 0.5X$) with no significant deviation from rectilinearity ($F < 1$) and no significant deviation

from/

from zero slope ($F < 1$).

Despite significant deviation from rectilinearity in experiment G, it is clear, from a consideration of the time relations of the increase in M.C.V. to the total follicular cell population in experiments A and G, that, in this system, cell hypertrophy does not precede hyperplasia but is concomitant with it.

DISCUSSION

These observations support those of Thyssen (1947) concerning the shape of the growth curve for weight of the rat thyroid under a goitrogenic stimulus. The existence of a lag phase lasting approximately 2 days, an exponential phase lasting 10 days and a plateau phase which persists for as long as the goitrogen is administered were all confirmed. The biphasic growth curve described by Crooks et al. (1964) was not observed. This can be accounted for by the long (weekly) intervals between their successive observations, which were not frequent enough to reveal the true form of the growth curve.

The time relations between the changes in mean cell volume and the increase in the follicular cell population suggest that the primary histological response of the rat thyroid follicular cells to this dose level of methylthiouracil is not hypertrophy as one might have anticipated. Cell hypertrophy goes on throughout the growth cycle and the mean cell volume does not reach its maximum until the plateau phase is reached when cell division has virtually ceased. This phenomenon may be accounted for by the fact that during the phase of exponential cell division there exists a mixed population consisting of cells preparing to divide and those newly divided. This leads one to differentiate between increase in cell size prior to division and "true hypertrophy" such as is found when cell division/

division has ceased in the plateau phase of cellular proliferation.

In view of these findings the statement of Crooks et al. (1964) that the increase in thyroid weight in the first stages of the goitrogenic response is principally due to cell hypertrophy and that follicular cell hyperplasia occurs mainly at a later stage has not been substantiated. It must be emphasised, however, that the conclusions with regard to the absolute values of the M.C.V. (μ^3) and the precise pattern of follicular cell hypertrophy must be regarded with some reserve because of the effect of fixatives on cell size referred to by Santler (1957) and also because the M.C.V. is a value derived from the R.C.V. and follicular cell concentration and therefore contains the errors inherent in both estimates.

The constancy of the mean follicular cell concentration throughout the growth cycle implies that this population, like the mean gland weight begins exponential growth after a lag of approximately two days and reaches a plateau around the twelfth day on methylthiouracil. One important consequence of this observation is that there is now available a population of normal mammalian cells in their normal (or nearly normal) environment undergoing exponential growth at a measurable rate. This model, therefore, has possible application to studies of cell population kinetics, biochemical "thermodynamic steady state" observations and the effects of various agents on cell division <u>in vivo</u>. It has been used/

used by me to study the effects of graded doses of x-rays on the thyroid follicular population of the rat in relation to the problems encountered in the treatment of thyrotoxicosis with ionising radiation. The results of these investigations are presented in Section II of this thesis.

SUMMARY

The goitrogenic effect of methylthiouracil was studied in the male Wistar rat. A growth curve for thyroid weight consisting of a lag phase lasting two days and an exponential phase of ten days duration terminating in a plateau phase is described. As the follicular cell concentration remains constant throughout the three phases of the growth curve, the total thyroid follicular cell population undergoes an identical pattern of increase to that for gland weight. Estimation of the mean follicular cell volume throughout the growth cycle suggest that, contrary to common supposition, cell hypertrophy does not precede hyperplasia in the goitrogenic response but is concomitant with it.

The demonstration that under a goitrogenic stimulus the thyroid follicular cells reproduce exponentially provides a model for quantitative studies of agents affecting cell division *in vivo*.

SECTION II

THE EFFECTS OF X-RADIATION ON GOITROGEN INDUCED GROWTH OF THE RAT THYROID

INTRODUCTION

The molecular events following bombardment of a tissue by ionising radiation are discussed by Bacq and Alexander (1961). When x-rays or γ-rays enter a tissue they eject electrons from the atoms by which they are absorbed. These ejected electrons in turn eject electrons from other atoms and are responsible for most of the ionisations which occur. In the case of β-irradiation electrons are again ejected with similar consequences. Charged particulate radiations such as α-rays and protons collide with bound electrons and eject them to produce ions. The ejected electrons once more go on to produce more ionisations. Neutrons interact to produce protons which behave as described above. Because of the "crossfire" of secondary particles for any dose of external radiation the absorbed radiation dose is greater at the centre of a solid organ compared to its periphery.

It should be noted that molecules can be damaged directly or indirectly by ionisation. Direct damage results when the molecule is ionised by the passage of an ionising particle through it. Indirect damage can also result if the molecules are dissolved in water which can be ionised to form free radicals which react with the molecules with consequent changes in their physico - chemical nature and function. In biological systems where water is the universal solvent indirect damage from free radicals is probably the most/

most important.

The biological effects of ionising radiation can be described in terms of damage to certain specific sites in the cell, i.e. the radiobiological target. If damage to any one of several sites results in loss of a particular function, then the radiobiological target is large and as hits are random events the chance of loss of function will be high even with small doses of radiation. If on the other hand only damage to one particular site will result in loss of function then the radiobiological target is small and larger doses of radiation will be required to achieve the same degree of inactivation. The amount of radiation required to achieve a particular effect is normally expressed in terms of the D_{37} dose, which is that dose of radiation required to achieve 63% inactivation (or 37% survival). The figure 37% is chosen because it is equal to $\frac{100}{e}$% where "e" is the natural logarithmic base with convenient mathematical properties. In considering reproductive integrity the D_{37} dose for any cell population is that which leaves 37% of the population reproductively intact; 63% of the cells are damaged and lose their ability to divide.

Doses of radiation can be measured in terms of the amount of radiation energy absorbed by the tissue in rads where 1 rad is equivalent to 100 ergs absorbed energy per gramme of tissue.

Very little quantitative information is available on the radio-

biology of organised tissues. Walters, Anson and Ivey (1931) found no histological changes in the thyroid glands of dogs treated with doses of x-rays similar to those used in the treatment of thyrotoxicosis at that time. Levene, Andrews and Kniseley (1955) studied the thyroidal effects of large doses of radioiodine (^{131}I) given to dogs. Following the administration of the dose of ^{131}I there was initial uptake of the isotope by the thyroid gland. From the fourth day after ^{131}I administration there was a rapid loss of radioactivity from the gland and a rise in the bound plasma radioactivity. By the twelfth day there was very little radioactivity left in the thyroid. These changes were explained by further histological and radicautographic observations. These revealed an initial patchy distribution of ^{131}I throughout the thyroid. No histological changes were observed during the first 3 days after radioiodine administration. By the sixth day there was massive necrosis in the central part of the gland from which nearly all radioactivity had been lost. However, the isotope was still concentrated in a peripheral rim of histologically intact follicles. From the twelfth day onwards even this rim of intact tissue had disappeared and the radioiodine was only concentrated in small islands of functioning cells. Clearly in this extremely complex dynamic situation no accurate assessment of radiation dose to any of the thyroid cells was possible. Similar studies by Findlay and Leblond/

Leblond (1948) and Goldberg, Chaikoff, Lindsay and Feller (1950) on rats and by Freedburg, Kurland and Blumgart (1952) in humans all yielded similar results.

In order to achieve accurate dosimetry, St. Aubin, Kniseley and Andrews (1955) studied the effects of external radiation (x-rays) on thyroid histology and ^{131}I metabolism in the dog. With a dose of 21,000 rads they found almost identical results to those obtained by Levene et al. (1955), i.e. accelerated release of ^{131}I (tracer dose) from the 4th day onwards accompanied by gross central necrosis and the same surviving rim of follicles at the periphery of the gland. This suggests a biological explanation for these phenomena, e.g. blood vessel necrosis resulting in tissue ischaemia. The latent period of 3 days with both ^{131}I and external radiation therapy before histological and functional changes occurred argues against the theory that in the case of ^{131}I, time is needed for the cumulative radiation dose to build up to lethal levels. Although the same authors observed similar changes with 10,000 rads they were surprisingly of minimal degree. This small histological and functional response to a massive dose (10,000 rads) of x-rays is also consistent with the electron microscope studies of McQuade, Seaman and Porporis (1956) on the rat thyroid in which no changes were found within 6 hours of a 17,200 rad dose or within 5 days of a 6,880 rad dose.

Skanse/

Skanse (1948) observed that the goitrogenic response of the chicken thyroid to TSH and thiouracil administration could be inhibited by large (50 μCi) but not by small (10 μCi) doses of radioiodine ^{131}I. Doniach and Logothetopoulos (1955) used ^{131}I to study the effects of ionising radiation on the rat thyroid. In rats simply given 30 μCi ^{131}I there was a decrease in thyroid weight and an increase in follicular cell height indicating that cell hypertrophy had occurred, presumably due to increased TSH stimulation secondary to radioiodine induced thyroid failure. The percentage uptake of a tracer dose of ^{131}I by these "compensated" glands was normal however. If the rats were hemithyroidectomised prior to treatment with ^{131}I there was again essentially normal uptake of a tracer dose of ^{131}I in the presence of even greater "compensatory" follicular cell hypertrophy than in the controls. Finally the normal goitrogenic response to propylthiouracil was decreased in the radiated group and was accompanied by a marked reduction in the mitotic index although cell hypertrophy was not reduced. Thus, doses of radiation which markedly reduced reproductive capacity did not impair the ability of the follicular cells to survive and hypertrophy and the capacity of the whole gland to function normally. The high incidence of abnormal nuclear forms in this and other studies suggested that the loss of ability to divide was due to chromosome damage.

The/

The work of Doniach and Logothetopoulos (1955) was extended by Crooks, Greig, Macgregor and McIntosh (1964) who measured the degree of inhibition of the goitrogenic response of the rat thyroid to methylthiouracil administration produced by various accurately measured doses of x-rays from an external source. They found progressive inhibition of the goitrogenic response with increasing doses of x-rays although the uptake of a tracer dose of ^{131}I was unaffected by doses of up to 1,600 rads. Histological examination of these glands was not carried out so that the contribution of cell division and cell hypertrophy to the total gland weight could not be assessed. However their observations were in keeping with those of Doniach and Logothetopoulos (1955) and their dose response curve for gland weight after 28 days on methylthiouracil after radiation was similar to those obtained by in vitro by Puck and Marcus (1956) and Hewitt and Wilson (1961).

In connection with the above observations, the work of Weinbren, Fitschen and Cohen (1960) is of interest. They performed hepatic radiation in the rat with doses of x-rays (5,000 rads) insufficient to cause immediate or delayed cell death. Many months later the animals were subjected to partial hepatectomy. When the irradiated cells tried to make good the functioning hepatic cell mass by cell division many of them underwent "mitotic death". In this experiment and that of Crooks et al. (1964) the radiation damage was therefore latent/

latent and was revealed only by the induction of cell division. However Al-Hindawi and Wilson (1965) demonstrated by titrated thymidine labelling studies that, in the rat thyroid, D.N.A. synthesis was decreased and cell half-life shortened by ionising radiation.

All these observations indicate that doses of radiation which markedly reduce reproductive capacity do not significantly impair the ability of the follicular cells to survive and hypertrophy and the capacity of the whole gland to function normally. This along with the demonstrations of cell life shortening (Al-Hindawi & Wilson, 1965) and mitotic death (Weinbren et al. 1960) provides an attractive explanation of the high hypothyroid rate in thyrotoxic patients treated with radioiodine therapy. In other words a dose of radioiodine sufficient to bring about a rapid decrease in thyroid function is likely to result in long term thyroid failure due to accelerated cell death and failure of cell replacement.

A study of relevant radiobiological observations <u>in vitro</u> casts further light on the possible sequelae of whole organ irradiation. Puck, Cieciura and Robinson (1958) put tissue culture on a new footing with the development of methods for maintaining mammalian cells <u>in vitro</u> whose chromosome structure, biochemical behaviour and genetic make-up were, as far as could be determined, identical to that of the cells in the tissue of origin. Using these cells,

Puck,/

Puck, Morkovin, Marcus and Cieciura (1957) were able to construct radiation dose-response curves for reproductive integrity for a variety of euploid human cells grown *in vitro*. Hewitt and Wilson (1961) reported their dose-response curves for various mouse leukaemia cells. These cells were irradiated *in vitro* but cultured *in vivo* by injection into test animals. The dose response curves for all the tumour cells tested by Hewitt & Wilson (1961) were very similar to one another and to those found by Puck and Marcus (1956), Puck et al. (1957), Morkovin and Feldman (1960) and Berry and Andrews (1961). The fact that similar quantitative responses were obtained by different investigators working with different cell types under different conditions argues that the radiobiological phenomena are of such a fundamental nature as to be applicable to mammalian cells irrespective of the site of origin providing variables such as the degree of oxygenation are eliminated (Hewitt, 1962).

It must be emphasised, however, that the situation is more complex than is indicated by the experiments quoted above which concern themselves, in the main, with the effects of radiation on one aspect of cell activity, i.e. the ability to divide an indefinite number of times (reproductive integrity). Sinclair (1961) reported the induction of cell mutants which survived but grew more slowly than the reproductively intact cells and unirradiated/

unirradiated controls. Thus after irradiation there exists a heterogeneous population with respect to cell division and this probably applies to every aspect of cell activity. This heterogeneity was also demonstrated by the experiments of Puck and Marcus (1956), Tolmach and Marcus (1960) and Tolmach (1961). They showed that cells damaged by radiation may lyse before division, grow without division to form giant cells which ultimately die or divide a limited number of times. In addition, Elkind, Han and Volz (1960) also found that many reproductively damaged cells could divide a limited number of times, the number being inversely related to the dose of x-rays.

The preceding discussion illustrates the complexity of the dynamic events which follow irradiation of the cell population of a whole organ. Each individual effect such as immediate cell death or loss of reproductive capacity will have its own dose response characteristics. In addition, as many radiation effects are probably inter-related in terms of combinations of genetic damage, as the radiation dose increases it is unlikely that the relative proportions of the various types of damaged cells will follow a simple pattern.

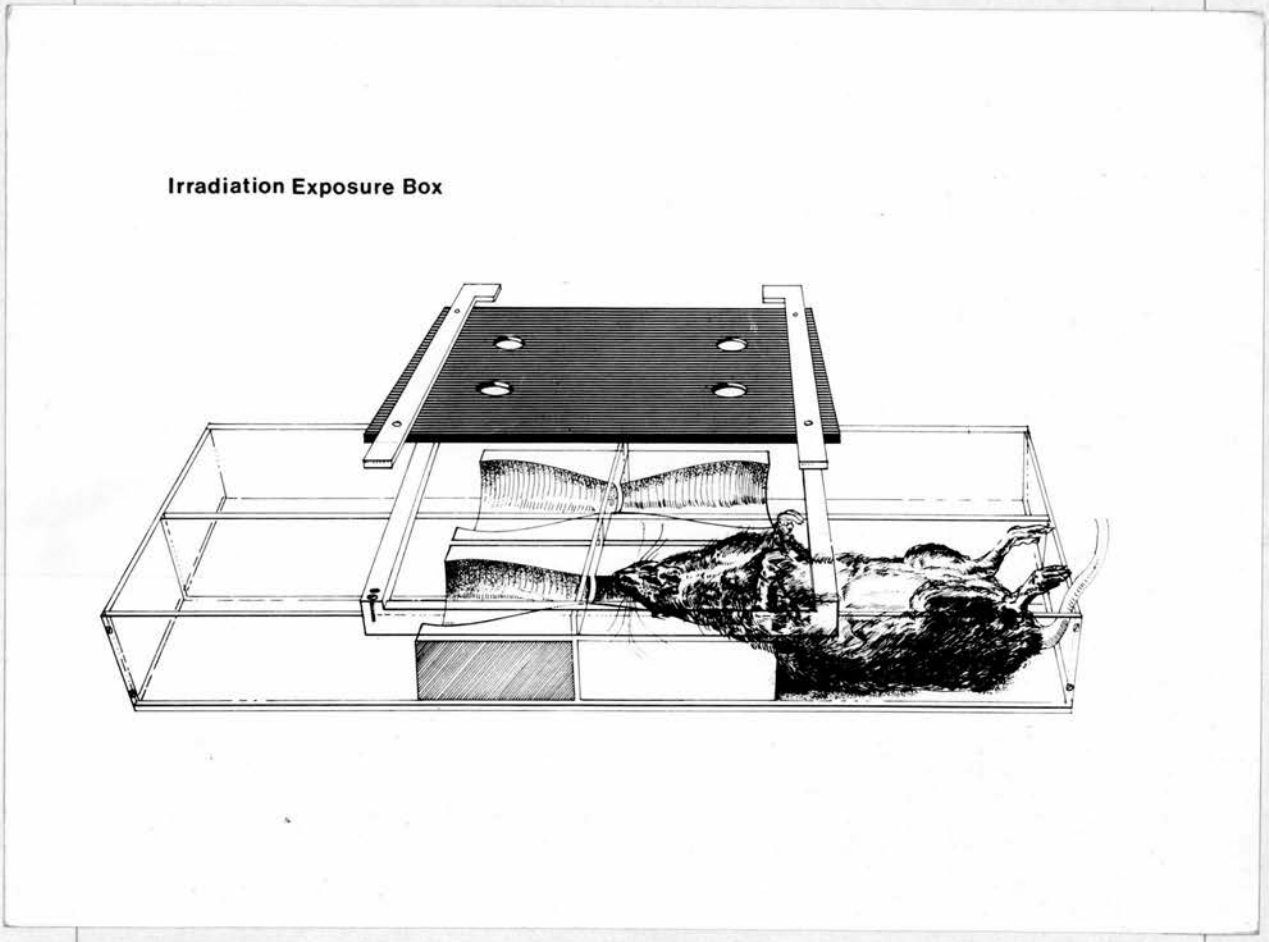
In the light of this, it was thought that the model described in the previous section in which the cells of the rat thyroid divide exponentially as a result of methylthiouracil administration would provide an interesting system for quantitative investigation of the effects of x-radiation on cell viability, function and reproductive capacity/

capacity *in vivo*. The goitrogenic response provides a population of normal mammalian cells in their "normal" environment undergoing predictable division at a constant and measurable rate. These experimental conditions have hitherto only been available in bacterial or tissue culture. By a study of the effects of graded doses of x-rays on the goitrogenic response it was hoped to assess the applicability of the *in vitro* observations quoted above to a strictly *in vivo* situation. In particular it was hoped to assess the possibility of a "radiation partial thyroidectomy" or its functional equivalent in mammals because of the relevance of this question to the treatment of hyperthyroidism with radioiodine (^{131}I).

Fig. 4

Method of Irradiating the Rat Thyroid

Fig. 4



Method of Irradiating the Rat Thyroid

MATERIALS AND METHODS

Doses of x-rays in the range 0 to 2,500 rads were delivered to the rat thyroid from a Westinghouse 250 KV machine with an output of 25 rads per minute obtained with 180 KV , 12.14 m.A. and a 0.5 mm. copper filter. The radiation dose delivered was monitored by an ionisation chamber set in the position occupied by the rat thyroid relative to the collimator of the x-ray machine. The rats were anaesthetised by an intraperitoneal injection of 1.5 to 3 mls. freshly prepared 2.5% tribromethylalcohol solution (Avertin) according to the length of time required for the radiation dose to be delivered. Four rats were irradiated at a time by means of the apparatus shown in Fig. 4. The rats were placed in the supine position with the points of their noses touching the transverse partition of the box. Their heads rested on pillows of "Mix-D wax" in order to bring about maximum back scatter and uniform radiation of the thyroid. The rats' bodies were shielded with a sheet of lead 3 millimeters thick which fitted the collimator of the x-ray machine and through which 4 holes had been made overlying the position of the rat thyroids. The position and size of the holes had previously been determined by transfixing dead rats through the centres of the holes with needles. Dissection of the rats' necks was then performed to determine the position of the thyroid in relation to the needle. Using this technique doses of 0, 200, 300, 400,/

400, 600, 800, 1,000, 1,500 and 2,500 rads were delivered to the thyroid region in different groups of adult male Wistar rats.

Fresh aliquots of 0.1% methylthiouracil in 1% sucrose were prepared every second day as described in Section I. In addition a suspension of methylthiouracil in 0.85% saline was prepared to a concentration of 25 mg/ml. Immediately following irradiation each animals received a subcutaneous injection of 1 ml (25 mg) of this suspension. This procedure ensured a uniform starting point for thyroid stimulation as many of the animals were drowsy during the first few hours after anaesthesia and could not be relied on to drink the 0.1% methylthiouracil solution with which their normal drinking water had been replaced. The suspension of methylthiouracil was preferred to the alkaline methylthiouracil solution which often produced skin necrosis after injection. Prior to each injection the methylthiouracil suspension was shaken in order to ensure uniform and consistent concentration from animal to animal.

In each radiation dose group random groups of animals were killed at intervals following irradiation, their thyroids dissected out, cleaned and weighed and estimates of the mean follicular cell concentration obtained as described in Section I.

EXPERIMENTAL DETAILS

Experiment G. Adult male Wistar rats weighing 220 to 280G were allocated at random into 68 groups of 7 each.

Ten/

Ten groups (i.e. 70 animals) were used to determine the normal (control) goitrogenic growth response curve. The animals were each given a subcutaneous injection of 1ml (25 mg) of the methylthiouracil suspension and their drinking water replaced by the 0.1% methylthiouracil solution. Two groups (14 animals) were killed on day zero and one group (7 animals) was then killed on days 2, 4, 6, 8, 10, 12, 14 and 16 after the commencement of methylthiouracil administration.

Thirteen groups (91 animals) received a dose of 200 rads to their thyroid glands following which 25 mg of methylthiouracil was injected subcutaneously and 0.1% methylthiouracil in 1% sucrose substituted for their drinking water. Two groups (14 animals) were killed on day zero and one group (7 animals) on days, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 after irradiation on methylthiouracil.

Twenty groups (140 animals) received a dose of 300 rads to their thyroids and methylthiouracil administered as above. Two groups (14 animals) were killed on day zero and one group (7 animals) on days 2,4,6, 8, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33 and 35 after irradiation on methylthiouracil.

Finally in this experiment 25 groups of animals (175 animals) received a dose of 400 rads to their thyroids and were started on methylthiouracil administration as above. Two groups (14 animals) were/

were killed on day zero and one group on days, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 35, 37, 39, 41, 43, 45 and 47 after irradiation on methylthiouracil.

Experiment H. In this experiment the general procedure was identical to that in Experiment G. The animals were once more constituted into random groups of 7 each. A control (non-irradiated) goitrogenic response was obtained by killing two groups (14 animals) on day zero and one group on days, 2, 4, 6, 8, 10, 12, 14 and 16 after commencing methylthiouracil administration. Sixteen groups (112 animals) received a dose of 600 rads to the thyroid, of which two groups (14 animals) were killed on day zero and one group (7 animals) on days 2, 4, 6, 8, 10, 14, 17, 20, 25, 28, 32, 35, 39 and 42 after irradiation on methylthiouracil. Eighteen groups (126 animals) received 800 rads to the thyroids, of which two groups were killed on day zero and one group on days 2, 4, 6, 8, 10, 14, 18, 21, 25, 28, 32, 35, 39, 42, 46 and 49 after irradiation on methylthiouracil. Finally 24 groups (168 animals) received 1,000 rads to their thyroids and were started on methyl-thiouracil in the standard way. Two groups were killed on day zero and one group on days, 2, 4, 6, 8, 10, 12, 16, 20, 23, 27, 30, 34, 37, 41, 44, 48, 51, 55, 62, 69, 92 and 132 after irradiation.

In addition in this experiment a further 24 random groups of 5 animals were used to determine the reproductive potential of the thyroid/

thyroid follicular cells at intervals up to one year after 1,000 rads x-radiation in the following way. Three months after irradiation 6 groups of animals (30 animals) received methylthiouracil in the usual way. One group of animals was killed at intervals of 0, 2, 4, 6, 10 and 12 days after commencing the drug. The growth pattern of the thyroid gland weight and thyroid follicular cell population was then compared to that obtained with methylthiouracil administration in the first 10 days after irradiation. An identical procedure was carried out at 6, 9 and 12 months after initial irradiation. Unfortunately, in this experiment many of the animals died so that in some cases the final numbers are not as great as those originally allocated. The actual number of animals finally examined are indicated in the results and in the Appendix (Table 20.)

Experiment J. In this experiment 18 groups of 6 animals each (108 animals) were randomly constituted. Six groups were used to obtain the control (un-irradiated) goitrogenic response growth curve. The animals were started on methylthiouracil in the usual way and one group killed on days 0, 2, 4, 6, 8 and 10 after commencing methylthiouracil. Another 6 groups were treated with 1,500 rads to their thyroids before commencing methylthiouracil administration. One group of these animals was killed on days 0, 2, 4, 6, 8 and 10 after/

after irradiation. The remaining 6 groups of animals received 2,500 rads to their thyroids, were started on methylthiouracil and one group killed on days 0, 2, 4, 6, 8 and 10 after irradiation. Pilot studies had shown that many animals receiving doses in the range 1,500 to 2,500 rads died 14-20 days after irradiation. Consequently these experiments were not prolonged beyond 10 days irradiation.

Selection of Sample Groups of Animals for Follicular Cell Concentration (F.C.C.) Estimations

As this series of experiments proceeded certain patterns were observed with regard to the effect of radiation on the goitrogenic response of the whole gland, the number of follicular cells per unit volume, the reproductive capacity of the follicular cells and the occurrence of cell death. As these patterns emerged it became clear that in order to confirm them over the whole radiation dose range, sampling of the follicular cell counts would be sufficient. Consequently estimates of the follicular cell concentration were carried out on the following groups only.

Experiment G. Follicular cell counts were carried out on all the animals in the 0 rad, 200 rad and 400 rad groups but in none of the 300 rad group.

Experiment H. Follicular cell counts were carried out on approximately half the groups of animals in the 0 rad, 800 rad and 1,000 rad/

1,000 rad groups. The actual groups in which counts were carried out is indicated in the results (Tables III, IV and V; Appendix Tables 8, 9, 10 and 11). No follicular cell counts were performed in the 600 rad group.

Experiment J. Follicular cell counts were carried out on all the animals in the 0 rad and 2,500 rad groups. No follicular cell counts were carried out on the 1,500 rad group.

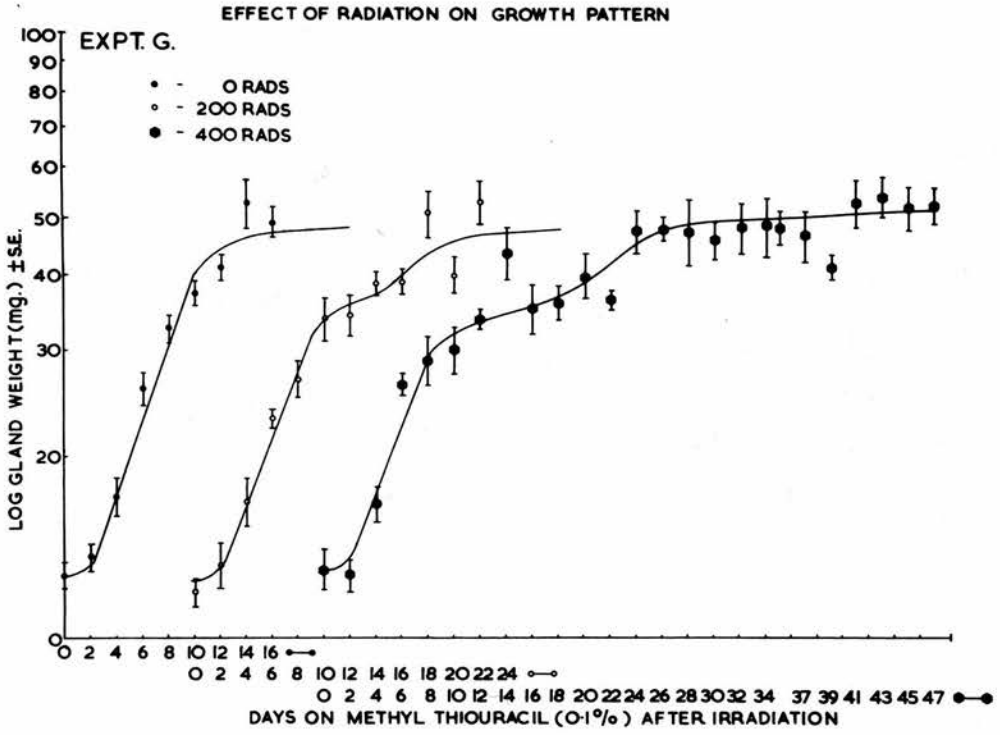
RESULTS

The Effects of X-Rays on the Goitrogenic Response

Experiment G. In this experiment the effects of doses of 200 rads and 400 rads x-irradiation on the goitrogenic response of the rat thyroid to methylthiouracil administration were observed. The results are contained in Table III (Appendix Tables 4, 5 and 7) and illustrated diagrammatically in Fig. 5, where the log of the mean gland weight is plotted against the number of days on methylthiouracil after irradiation. In the unirradiated (control) goitrogenic response there is the usual lag phase of approximately 2 days followed by exponential growth for 8 to 10 days until the plateau is reached.

In the radiated groups the pattern of a lag phase of 2 days followed by initial exponential growth identical to that in the control goitrogenic response was observed. However, as a result of x-irradiation it can be seen that the exponential growth phase is interrupted by a pause during which there is little or no increase in gland weight. The pause is temporary, however, and is terminated by a resumption of growth until the plateau weight attained by the controls is reached. It should be noted that the pause occurs earlier and lasts longer in the 400 rad group compared to the 200 rad group. As an approximation arrived at by eye the pauses in exponential growth in the 200 rad and 400 rad groups began at 33mg and/

Fig. 5



The Effects of 200 rads and 400 rads X-radiation on the Goitrogenic Response to Methylthiouracil Administration
The log mean gland weight ± standard error (S.E.) is plotted against time on methylthiouracil after irradiation.

TABLE III: Experiment G

Effects of X-Irradiation of the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid

Days After Irradiation on Methyl-Thiouracil	Mean Gland Weight (mg.) ± Standard Error			Mean Number of Cells/10mm^3 ± Standard Error		
	0 rads	200 rads	400 rads	0 rads	200 rads	400 rads
0	12.7±0.7	11.9±0.6	13.0±1.0	3.68±0.13	4.81±0.58	4.05±0.21
2	13.7±0.7	13.1±1.1	12.7±0.8	3.74±0.14	3.56±0.20	3.94±0.17
4	17.1±1.2	16.8±1.5	16.7±1.1	3.43±0.19	3.38±0.19	3.45±0.11
6	24.6±1.4	23.2±0.8	26.3±0.11	3.68±0.11	3.47±0.32	3.62±0.33
8	32.5±1.8	26.9±1.9	28.8±2.7	3.35±0.20	3.24±0.15	3.33±0.10
10	37.2±1.7	33.7±2.6	30.0±2.6	3.55±0.14	3.47±0.12	3.53±0.13
12	41.2±2.0	34.3±2.7	33.5±1.0	3.32±0.11	3.63±0.17	3.70±0.16
14	52.5±4.9	38.5±1.7	43.2±4.4	3.54±0.18	3.72±0.08	3.16±0.13
16	49.0±2.5	38.8±1.9	35.0±3.1	3.42±1.1	3.69±0.11	3.87±0.13
18		50.2±4.3	35.7±2.2		3.42±0.17	3.81±0.16
20		40.0±2.8	39.5±3.4		3.75±0.07	3.62±0.12
22		52.4±4.5	36.1±1.5		3.68±0.18	3.90±0.10
24			46.9±3.8			3.76±0.16
26			47.4±2.0			3.51±0.12
28			46.9±5.7			3.48±0.11
30			45.3±3.3			3.64±0.19
32			47.7±4.5			3.49±0.13
35			47.8±3.2			3.38±0.12
37			46.2±4.6			3.56±0.14
39			41.0±2.0			3.66±0.18
41			52.1±4.6			3.66±0.18
43			53.5±4.1			3.60±0.12
45			51.1±4.2			3.40±0.10
47			51.9±3.6			3.32±0.06
Regression Equation Y = a + bx				Y = 3.57-0.01x	Y = 3.55+0.00x	Y = 3.77-0.01x
Analysis of Variance of Regression	F Ratio for $b \neq 0$			< 1	< 1	< 1
	F Ratio for Deviation from Rectilinearity			< 1	< 1	< 1

and 30 mg respectively. The plateau weight was finally attained at between 14 and 18 days in the 200 rad group and between 20 and 24 days in the 400 rad group.

Experiment H. In this experiment the effect of doses of 600 rads, 800 rads and 1,000 rads on the goitrogenic response were studied. The results are contained in Table IV (Appendix Tables 8, 9, 10 and 11) and shown diagrammatically in Fig. 6 where the log of the mean gland weight is plotted against days on methylthiouracil after irradiation. As in Experiment G there is a lag phase of approximately 2 days followed by exponential growth at a rate similar to that found in the controls. Once more, the pause in exponential growth occurred earlier and lasted longer with increasing doses of x-rays. In the 1,000, 800 and 600 rad groups the pause commenced at gland weights of approximately 27 mg, 25 mg and 22 mg respectively. In the 600 rad group the plateau weight was reached at between 20 and 25 days after irradiation and in the 800 rad group at between 40 and 42 days. In the 1,000 rad group, as far as could be determined, the pause continued even up to 132 days after irradiation on methylthiouracil.

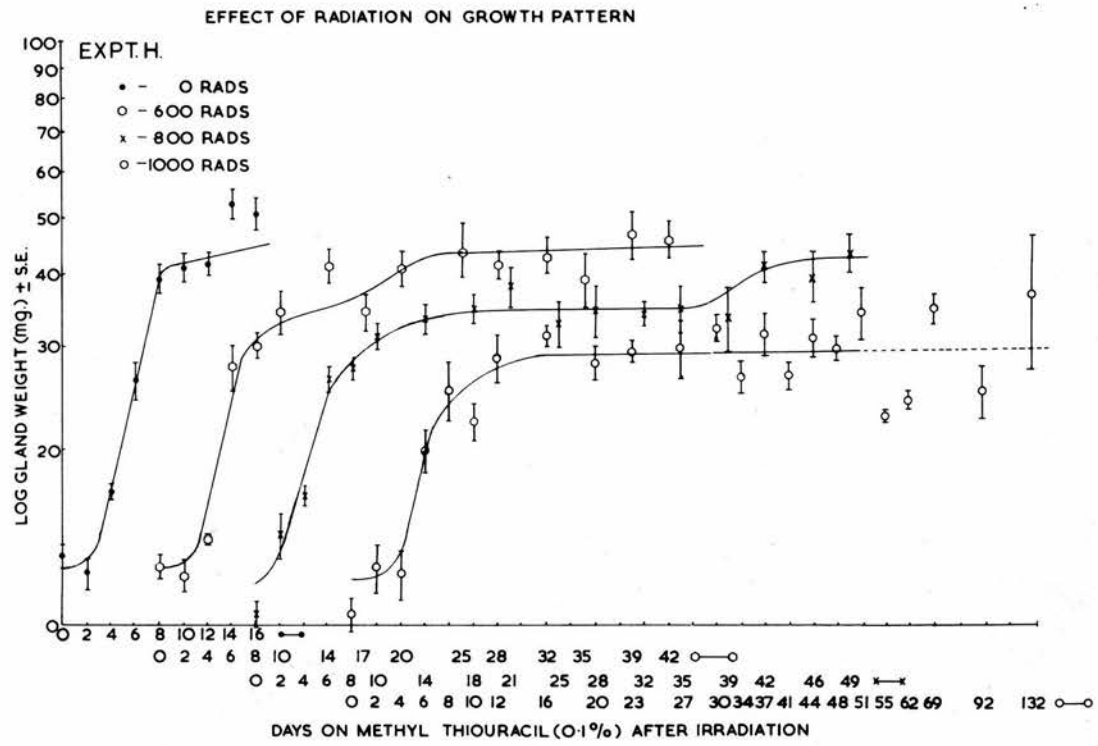
Experiment J. In this experiment the effects of doses of 1,500 and 2,500 rads on the goitrogenic response were studied. As mentioned previously these experiments were continued for only 10 days after irradiation because many of the animals treated with doses of this order/

TABLE IV: Experiment H

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid

Days After Irradiation on Methyl-Thiouracil	Mean Gland Weight (mg.) ± Standard Error				Mean Number of Cells/$10mm^3$ ± Standard Error		
	0 rads	600 rads	800 rads	1000 rads	0 rads	800 rads	1000 rads
0	13.1±0.6	12.6±0.6	10.4±0.5	10.4±0.7	4.42±0.10	4.12±0.15	3.99±0.17
2	12.3±0.8	12.1±0.7	14.1±1.3	12.5±1.2	3.82±0.15	3.79±0.10	3.47±0.13
4	16.9±0.5	14.0±1.2	16.5±0.7	12.8±1.2	3.25±0.03	3.55±0.08	3.85±0.28
6	26.2±2.0	27.5±2.5	26.2±1.2	19.8±1.7	3.50±0.15	3.63±0.13	
8	39.0±2.2	30.0±1.5	27.2±1.2	25.1±2.9			3.06±0.12
10	40.8±2.3	34.2±2.8	31.0±1.6	22.2±1.6	3.65±0.09		
12	41.4±1.9	41.0±2.8		28.5±2.7			3.39±0.18
14	52.3±3.1	34.1±2.4	33.3±1.9		3.77±0.21	3.71±0.10	
16	50.5±3.4			31.0±1.3			
17		40.6±2.7					
18			34.7±2.1				
20		43.9±4.4		28.0±2.0			
21			37.7±2.8				
23				29.3±1.3			3.44±0.11
25		41.1±2.2	32.6±2.8			3.70±0.10	
27				29.6±3.4			
28		42.9±3.0	34.4±3.4				
30				32.1±1.7			
32		38.8±4.1	33.9±1.5				
34				26.5±1.7			3.64±0.13
35		46.1±4.3	34.6±3.3			3.77±0.10	
37				31.4±2.6			
39		45.4±3.3	33.6±4.1			3.55±0.16	
41				26.6±1.4			3.59±0.09
42			41.1±2.2			3.78±0.60	
44				30.9±2.3			3.65±0.07
46			39.6±4.0			3.40±0.15	
48				29.6±1.5			
49			43.4±3.2			3.71±0.20	
51				34.3±3.5			
55				22.7±0.6			4.06±0.15
62				24.2±0.8			
69				34.8±2.1			3.52±0.10
92				25.1±2.6			
132				36.8±9.4			3.50±0.10
Regression Equation $Y = a + bx$					$Y = 4.05-0.04x$	$Y = 3.87-0.01x$	$Y = 3.94-0.00x$
Analysis of Variance of Regression	F Ratio for $b \neq 0$				2.0($p>0.05$)	1.2($p>0.05$)	<1
	F Ratio for Deviation from Rectilinearity				6.1($p<0.01$)	<1	<1

Fig. 6



The Effects of 600 rads, 800 rads and 1,000 rads X-irradiation on the Goitrogenic Response to Methylthiouracil Administration. The log mean gland weight ± standard error (S.E.) is plotted against time on methylthiouracil after irradiation.

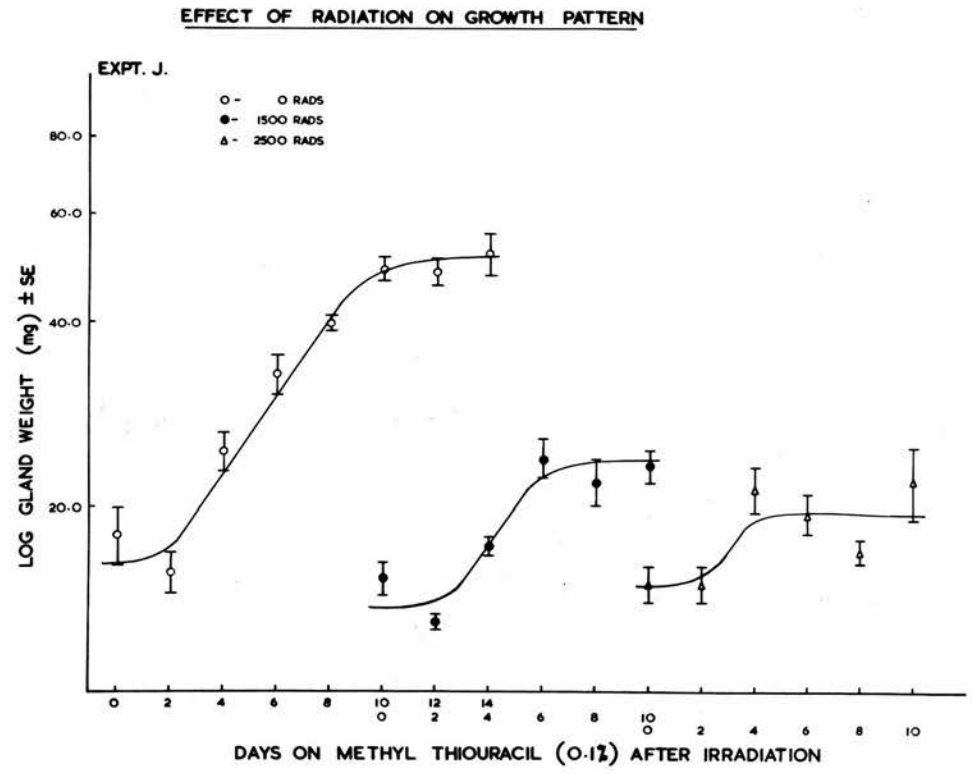
order died 14 to 20 days after radiation as a result of pharyngitis and pneumonia. The results are contained in Table V (Appendix Tables 12, 13 and 14) and shown graphically in Fig. 7. Once more the pattern is consistent with a lag phase of approximately 2 days and initial exponential growth in gland weight similar to that of the unirradiated controls. The evidence suggests that in this case the pause occurred in gland weight of 17 mg in the 2,500 rad group and 20 mg in the 1,500 rad group. Clearly no observations on the length of the pause in exponential growth could be obtained.

TABLE V: Experiment J

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid

Days After Irradiation on Methylthiouracil		Mean Gland Weight (mg.) ± Standard Error			Mean Number of Cells/10mm^3 ± Standard Error	
		0 rads	1,500 rads	2,500 rads	0 rads	2,500 rads
0		18.0±1.9	15.2±0.9	15.0±1.0	3.86±0.17	4.04±0.25
2		15.6±1.2	13.0±0.2	15.0±1.0	3.80±0.18	4.11±0.10
4		24.6±1.8	17.2±0.4	21.4±1.8	3.61±0.21	3.84±0.12
6		32.8±2.4	23.9±1.7	19.5±1.4	3.83±0.13	4.20±0.23
8		39.7±0.9	21.9±1.9	16.9±0.7	3.66±0.16	3.99±0.24
10		48.8±2.1	23.2±1.4	22.0±3.0	3.74±0.24	3.53±0.11
12		48.2±2.4			3.59±0.15	
14		51.5±3.9			3.67±0.22	
Regression Equation $Y = a + bx$					$Y = 3.82 - 0.01x$	$Y = 4.13 - 0.03x$
Analysis of Variance of Regression	F Ratio for $b = 0$				< 1	< 1
	F Ratio for Deviation from Rectilinearity				< 1	1.3 ($p > 0.05$)

Fig. 7

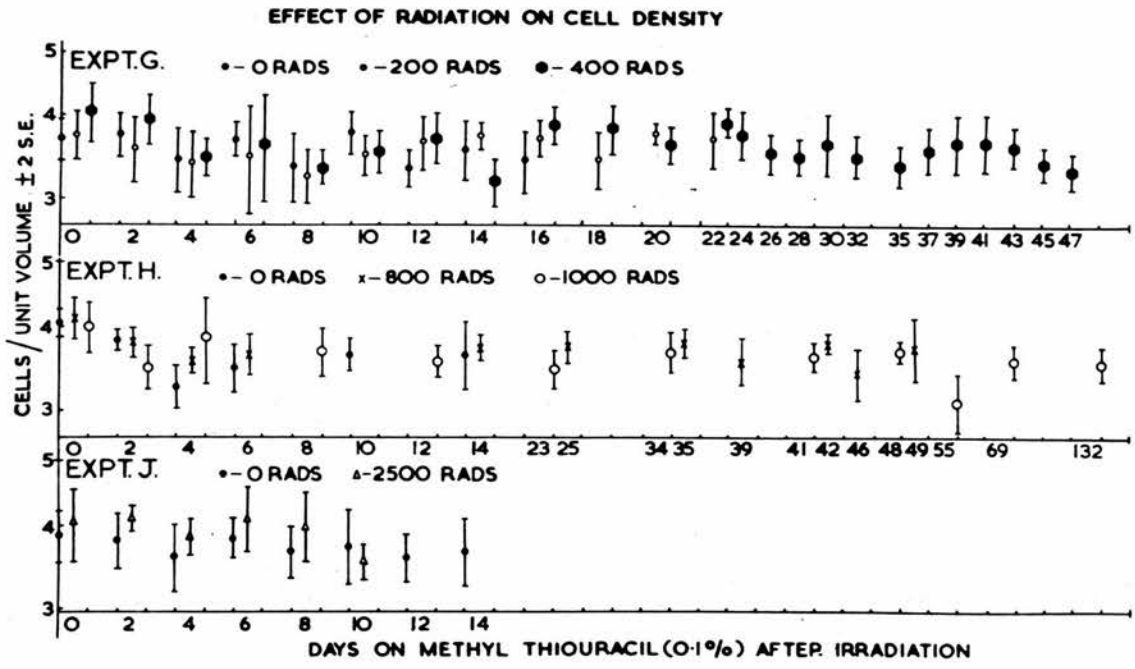


The Effects of 1,500 rads and 2,500 rads X-Irradiation on the Goitrogenic Response to Methylthiouracil Administration. The log mean gland weight ± standard error (S.E.) is plotted against time on methylthiouracil after irradiation.

The Effects of X-Irradiation on the Mean Follicular Cell Concentration

For purposes of presentation the results of Experiments G, H and J are best considered together. The results are contained in the respective parts of Tables III, IV and V (Appendix Tables 4, 5, 7, 8, 10, 11, 12, 13 and 14) and are shown diagrammatically in Fig.8 where the numbers of follicular cells per 10 mm^3 in each experiment are plotted against time after irradiation on methylthiouracil. In each case the regression equation ($Y = a + bx$) of mean follicular cell concentration on time after irradiation on methylthiouracil was calculated and the results shown in Tables III, IV and V. In each radiation group (200, 400, 800, 1,000 and 2,500 rads) an analysis of variance was performed on the regression equation and the values of the "F ratios" for rectilinearity and slope are also contained in Tables III, IV and V. These analyses revealed that, irrespective of the dose of x-radiation or time after irradiation on methylthiouracil there was no significant deviation from rectilinearity and that in all cases the slopes of the regression equations (b) did not differ significantly from zero. Therefore with doses of x-rays in the range 200 to 2,500 rads there was no significant change in the mean number of follicular cells per unit volume following goitrogenic challenge with methylthiouracil. A further analysis of variance was performed on all the data from Experiments G, H and J in order to determine the regression of the mean/

Fig. 8



The Effect of X-Radiation in the range 200 to 2,500 rads on the Follicular Cell Concentration of the Rat Thyroid during the Goitrogenic Response to Methylthiouracil Administration. The mean follicular cell concentration ± 2 standard errors (2 S.E.) is plotted against time after irradiation on methylthiouracil.

mean follicular cell concentration on radiation dose. This demonstrated that the regression equation $Y = 3.72 + 0.0001X$ had no significant slope ($F < 1$) and showed no significant deviation from rectilinearity ($F = 3.6$; $p > 0.05$). Therefore doses of x-rays in the range of 0 to 2,500 rads did not affect the mean follicular cell concentration.

It can therefore be concluded that for the conditions observed in these experiments the mean follicular cell concentration was constant, irrespective of time on methylthiouracil, dose of and time after x-radiation in the range 200 to 2,500 rads. Consequently the changes in gland weight in Experiments G, H and J parallel the changes in the mean total follicular cell population of the rat thyroid.

The Long Term Effects of 1,000 rads X-radiation on the Goitrogenic Response of the Rat Thyroid.

As part of Experiment H the goitrogenic effect of methylthiouracil (0.1%) was observed at intervals of 0, 3, 6, 9 and 12 months after 1,000 rads x-irradiation and estimates of the follicular cell concentration in each group were made as before. The results are shown in Table VI (Appendix Tables 15, 16, 17, 18 and 19).

The mean thyroid weight obtained after 10 days on methylthiouracil administration was observed at 0, 3, 6, 9 and 12 months and compared to that in the control (non-irradiated) goitrogenic response. In the non-irradiated group a mean thyroid weight of 48.8 ± 2.1 mg (mean ± standard error) was obtained. Goitrogenic challenge immediately after 1,000 rads x-irradiation (zero months) resulted in a mean gland weight of 22.2 ± 1.6 mg.; 3 months after irradiation 20.1 ± 1.6 mg.; 6 months after irradiation 19.3 ± 1.4 mg.; 9 months after irradiation 23.4 ± 4.6 mg.; 12 months after irradiation 16.7 ± 0.8 mg. The regression of gland weight after 10 days on methylthiouracil on time after x-irradiation was calculated and found to be $Y = 22.6 - 0.4X$. Analysis of variance of this regression equation revealed that it had zero slope ($F < 1$) and had no significant deviation from rectilinearity ($F = 3.7$: $0.01 < p < 0.05$).

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TABLE VI

Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil Administration at Intervals after 1000 rads x-irradiation

Days on Methyl-Thiouracil	0 rads		Months after 1000 rads x-irradiation to Thyroid Gland									
	Gland Wt. (mg.) ± S.E.	Mean No. follicular cells/10mm³ ± S.E.	Gland Weight (mg.) ± S.E.					Follicular Cells (10^6)/10mm³ ± S.E.				
			0	3	6	9	12	0	3	6	9	12
0	18.0±1.9	3.86±0.17	10.4±0.7	10.9±1.6	14.3±1.8	11.4±0.8	17.1±6.3	3.99±0.17	4.05±0.18	4.15±0.29	4.34±0.56	3.77 ±0.27
2	15.6±1.2	3.80±0.18	12.5±1.2	15.2±0.2	15.7±1.3	12.7±0.7	12.4±1.9	3.47±0.13	3.63±0.33	4.10±0.28	3.99±0.16	4.23±0.40
4	24.6±1.8	3.61±0.21	12.8±1.2	17.2±2.5	17.7±1.7	16.7±1.1		3.85±0.28	4.09±0.29	3.96±0.23	3.49±0.23	
6	32.8±2.4	3.83±0.13	19.8±1.7	19.0±1.6	21.2±2.0	19.4±1.3	14.2±0.6		3.53±0.17	3.70±0.32	3.77±0.32	3.86±0.27
8	39.7±0.9	3.66±0.16	25.1±2.9		20.3±1.4	18.1±0.7		3.06±0.12		3.67±0.23	3.87±0.04	
10	48.8±2.1	3.76±0.24	22.2±1.6	20.1±1.6	19.3±1.4	23.4±4.6	16.7±0.8		4.06±0.29	4.09±0.29	3.45±0.25	3.46±0.23
12	48.2±2.4	3.59±0.15	27.5±2.7	18.9±1.0			19.4±1.8	3.39±0.18	4.18±0.44			4.06±0.31
Regression Equation $Y = a + bx$								$Y = 3.94-0.00x$	$Y = 3.85+0.02x$	$Y = 4.05-0.02x$	$Y = 4.14-0.07x$	$Y = 3.92-0.01x$
Analysis of Variance of Regression	F Ratio for $b \neq 0$							< 1	<1	<1	<1	<1
	F Ratio for Deviation from Rectilinearity							<1	<1	<1	<1	<1

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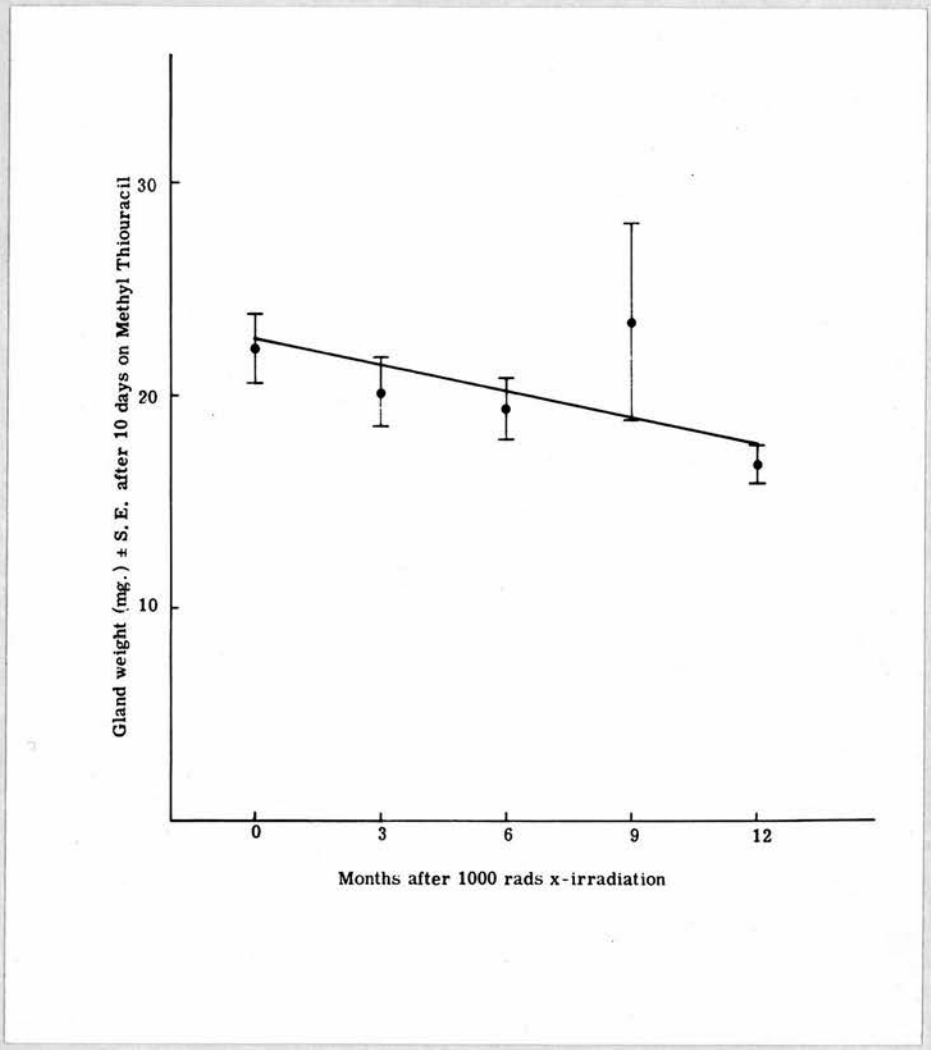
Fig. 9



Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil Administration at Intervals of 0, 3, 6, 9 and 12 months after 1,000 rads x-irradiation.

The regression of the mean follicular cell concentration on time on methylthiouracil was calculated for each goitrogenic challenge at the various intervals after initial irradiation and the results shown in Table VI. In each case the regression equation showed no deviation from rectilinearity ($F < 1$) and no regression significant deviation from zero slope ($F < 1$).

As the mean follicular cell concentration is constant irrespective of the time after irradiation and of time on methylthiouracil it can once more be concluded that the changes in gland weight found in the goitrogenic response parallel the changes in the total thyroid follicular cell population. Consequently over the period of study (1 year) there was no statistically significant diminution in the reproductive capacity of the thyroid follicular cells. Such a diminution had been anticipated in view of the current concept of "normal cell life span and cell turnover". According to the concept of "natural" cell death the remaining cells have to divide to make good this loss. In the case of thyroid which had received 1,000 rads x-radiation this would have meant using up some of the limited reproductive capacity left in the gland. This in turn should have reflected itself in a progressive reduction in the goitrogenic response over the 12 months following irradiation. It can therefore be concluded that within the limits of/

of resolution of this experimental technique there was no evidence of statistically significant turnover in the rat thyroid follicular cell population in the year following 1,000 rads x-irradiation of the thyroid.

Radiation Dose-Response Curve for Rat Thyroid Follicular Cells IN VIVO

The normal goitrogenic response of the rat thyroid to methylthiouracil administration reaches a plateau in approximately 10 to 12 days. This plateau is attained as a consequence of exponential growth brought about by endogenous TSH stimulation and presumably the plateau weight represents that mass of functioning thyroid tissue required to maintain euthyroidism in the face of methylthiouracil administration. Alternatively some factor regulating the upper limits for thyroidal volume may be operating. Whatever the total explanation for this phenomenon a plateau which is relatively constant from one experiment to another is reached after exponential growth which probably reflects a maximal division rate on the part of the thyroid cells.

In general terms, as the dose of x-radiation to the thyroid increases the number of cells capable of division will decrease. It can therefore be seen that in the irradiated groups the time taken to attain the plateau weight will be inversely proportional to the number of cells left immediately after irradiation which are capable of division (see Fig. 13). This simple concept has to be modified in the light of the results of Sinclair (1964) who demonstrated that the effect of radiation on cell reproductive capacity was not an all or none phenomenon but that clones of cells were/

were produced which were capable of cell division at a slower rate than the unirradiated controls. In view of this, the original statement that the number of reproductively intact cells left after radiation is inversely proportional to the time taken to attain the plateau weight has to be modified to say that with any radiation dose the number of reproductively intact cells cannot exceed that number which growing exponentially at a rate similar to the unirradiated controls would attain the plateau weight in the specified number of days. If these assumptions are correct, extrapolation backwards to the ordinate (log scale) from the time at which the plateau weight is achieved, with a straight line of slope identical to that found in the normal goitrogenic response, would give an estimate of the number of reproductively intact cells in existence immediately after irradiation. Several points about this procedure must be emphasised. Firstly the model being used here is not a good method for constructing radiation dose-response curves of this type. This is because of the variation in the response between different animals in any one group which makes a precise determination of the day on which the plateau weight is attained impossible. In order to improve the resolution even greater numbers of animals would have to be employed. The objection to this can be seen from the fact that in this series of experiments a total of 2,500 male Wistar rats were used over a period of 2 years and the number of animals which could/

could be housed at any one time was limited. Secondly it will be clear from an examination of Figs. 5, 6 and 7 that the period from the beginning of the pause in exponential growth to the growth spurt to the plateau weight is the least reliable part of a rather complex response to the radiation. This is in contrast to the simpler response reflected in the first part of the growth curve up to the pause in exponential growth. Thirdly the estimate of the number of reproductively intact cells immediately after irradiation is an estimate of the upper limit of the number of reproductively intact cells at that time. This is because the goitrogenic response after irradiation is probably distorted by slow growing clones of cells of the type described by Sinclair (1964) and by cells capable of limited division of the type described by Elkind, Han and Voltz (1963). Clearly interference by these types of cells will be most important in the low radiation dose groups where the plateau weight is attained relatively early and consequently the "distortion" produced by these "reproductively damaged" cells will be great. In the large radiation dose groups where the delay in the attainment of the plateau weight is much greater the contribution and therefore the distorting effect of these "reproductively damaged" cells will become progressively less. Unfortunately it is precisely in these large dose groups where the largest number of animals are required in order to observe the goitrogenic response over a long period of time/

time and the logistic objection outlined above becomes prohibitive. Finally the "doubling time" (time for one complete cell division cycle) is not absolutely constant from one experiment to another but varies in the range 4 to 6 days. In extrapolating back to the ordinate this variable adds another uncertainty to the estimate of the number of reproductively intact cells present immediately after irradiation.

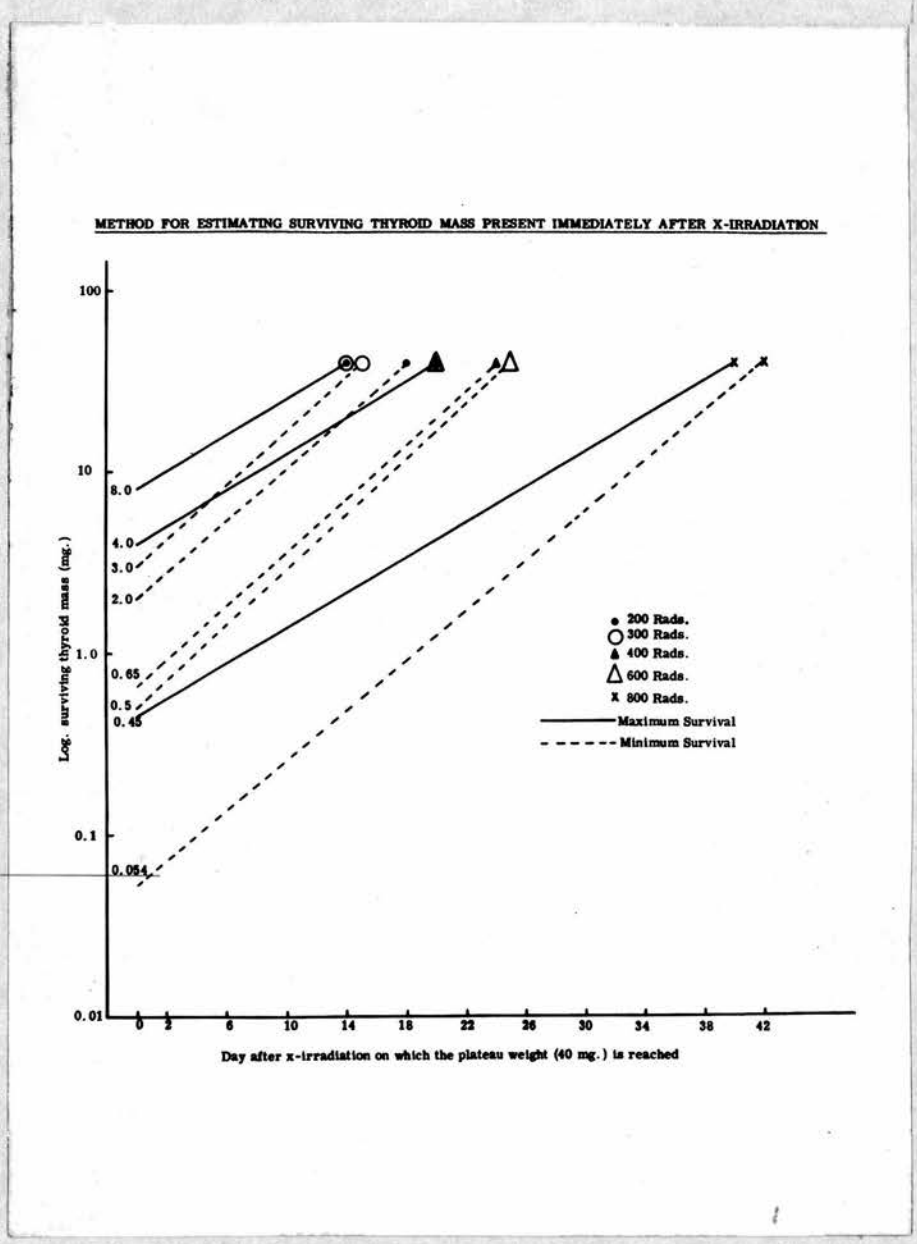
In spite of these objections it was thought worthwhile to attempt to obtain a radiation dose-response curve for reproductive integrity because the model provided an opportunity to study normal mammalian cells in their normal environment. Also, as mentioned in the introduction, the dose-response curves obtained by Puck et al. (1957) working with cells in tissue culture and Hewitt and Wilson (1961) with mammalian tumour cells irradiated in vitro and grown in vivo were remarkably similar. There is, therefore, a growing suspicion that the dose response characteristics for reproductive integrity are identical in all mammalian species (Hewitt, 1962) and this series of experiments provided an opportunity to test this view.

In order to obtain a dose-response curve which took account of the unavoidable variables inherent in this system the following procedure was adopted. A time range within which the irradiated thyroid gland weight reached the plateau weight attained by the controls (40 mg.) was derived by inspection of the growth curves for each/

Fig. 10

See Text for Explanation of Diagram

Fig. 10



See Text for Explanation of Diagram

each radiation dose group. This range was judged to be 14 to 18 days for the 200 rad group, 14 to 15 days for the 300 rad group, 20 to 24 days for the 400 rad group, 20 to 25 days for the 600 rad group and 40 to 42 days for the 800 rad group. By extrapolating back to the ordinate from the lower end of these ranges with a straight line with the least possible slope (i.e. corresponding to a doubling time of 6 days) an estimate of the upper limit of follicular cell survival was obtained for each x-ray dose level (Fig. 10). Similarly by extrapolating back to the ordinate from the upper end of these ranges with a straight line of maximum possible slope (i.e. corresponding to a doubling time of 4 days) an estimate of the lower limit of follicular cell survival was obtained for each x-ray dose level (Fig. 10). This procedure yielded the percentage survival ranges shown in Table VII. These results are shown diagrammatically in Fig. 11, alongside the dose-response curve for CBA mouse leukaemia cells obtained by Hewitt (1962). As mentioned previously the dose-response curves obtained by Hewitt are indistinguishable from those obtained in vitro by Puck and Marcus (1956), Puck et al. (1957) and Berry and Andrews (1961). These have come to be known under the collective term of the Puck-Hewitt Dose-Response Curve. It is clear from Fig. 11 that the Puck-Hewitt curve lies within the upper and lower limits for percentage survival of thyroid follicular cells in the dose range 0 to 800 rads.

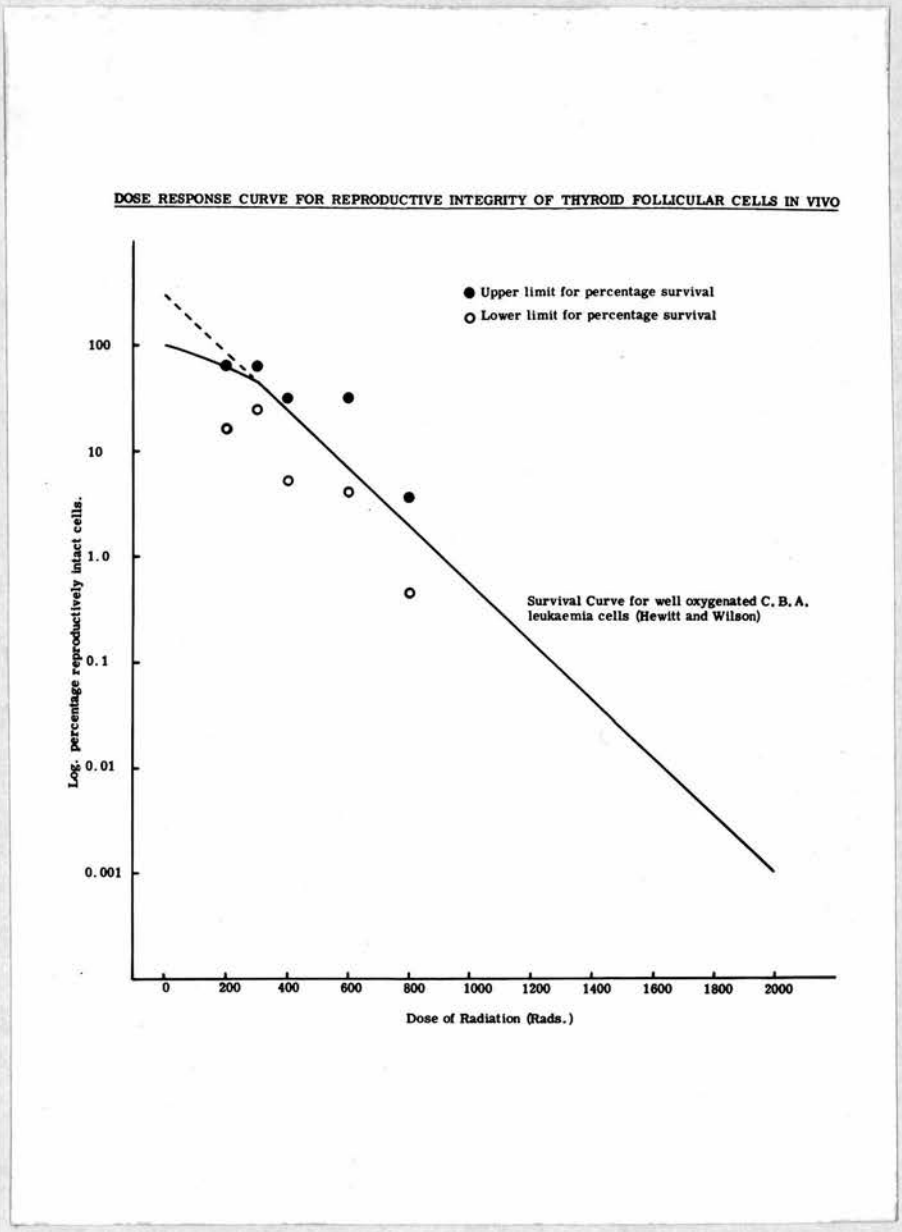
Despite/

TABLE VII

Minimum and Maximum Thyroid Mass Survival Following X-Irradiation

Doses of x-rays (rads)	Time Range in which Plateau Weight (40mg.) is reached (days)		Limits of Survival Expressed as Gland Weight (mg.)		Limits of Survival Expressed as Percentage of Initial Gland Weight (12 mg.)	
	Lower	Upper	Lower	Upper	Lower	Upper
200	14	18	2mg.	8mg.	17%	66%
300	14	15	3mg.	8mg.	25%	66%
400	20	24	0.65mg.	4mg.	5.4%	33%
600	20	25	0.5mg.	4mg.	4.2%	33%
800	40	42	0.054mg.	0.45mg.	0.46%	3.7%

Fig. 11



Comparison of the post-irradiation reproductive survival of rat thyroid follicular cells *in vivo* with the Puck-Hewitt Dose-response curve.

Despite the limitations of the technique outlined in the preceding paragraph the results are therefore consistent with the concept that the radiation dose response curves for reproductive integrity are similar for all mammalian cells providing they are well oxygenated. It also provides some further justification for the extrapolation of existing *in vitro* and *in vivo* radiobiological studies to human radiotherapeutic problems such as the treatment of thyrotoxicosis with ionising radiation.

Mathematical Models for the Effect of Ionising Radiation on the Follicular Cell Population of the Rat Thyroid.

On consideration of the possible dynamics of the thyroid follicular cells following irradiation it was felt that some convenient mathematical model might be obtained which would fit the observed phenomena and enable more reliable conclusions to be reached from the raw data.

Two patterns of cell division have been considered and will be called Model I and Model II respectively. For both models it is assumed that the effect of suppressing thyroid hormone will, for a normal thyroid, lead to an increase in the number of cells M according to

$$\frac{dM}{dt} = \lambda M \text{ so that } M_t = e^{\lambda t} \cdot M_o$$

Where M_o is the number of cells at time 0, M_t the number of cells at time "t" after commencing methylthiouracil, e the natural logarithmic base and λ a division constant.

From this we can deduce the generation time (i.e. time required for the cell population to double itself) as given by $T = \frac{\log_e 2}{\lambda}$. It is also assumed that over the time span of the experiment there is no cell mortality due to causes other than the original dose of radiation.

Model I/

Model I

We assume that the effects of radiation are such that the original cell mass M_0 is divided into:

P cells which escaped radiation damage and will behave as cells of a non-irradiated gland;

Q cells which are damaged, but can reproduce. For these cells, division may result in live cells of type Q, in live cells of type R (described below) and in dead cells (D) with probability θ_1, θ_2, and θ_3 respectively ($\Sigma\theta = 1$)

R cells which are damaged and cannot reproduce

D cells which are killed at the time of irradiation

P, Q and R cells are capable of producing thyroid hormone.

Thus after irradiation the active cell mass becomes:-

$$M_0 - D = N_0 = P_0 + Q_0 + R_0$$

Where Mo, Po, Qo and Ro are the number of M, P, Q and R cells at time zero and No = (Mo - D).

It was assumed for the sake of simplicity that after the administration of the hormone suppressing drug, the division of all cells capable of reproduction is governed by the same constant (λ) which is applicable to non-irradiated cells.

The total cell population at time "t" after irradiation on methylthiouracil/

methylthiouracil (N_t) therefore given by

$$N_t = P_t + Q_t + R_t$$

Where P_t, Q_t and R_t are the number of P, Q and R cells at time "t".

Now $$\frac{dP}{dt} = \lambda P$$

$$\therefore P_t = e^{\lambda t} P_o$$

It should be remembered that the division of the Q cells can result in the formation of Q cells or R cells or D cells with probability θ_1, θ_2 and θ_3 respectively and that the formation of Q cells results in an increase in the total Q cell population whereas the formation of R or D cells decreases it.

$$\therefore \frac{dQ}{dt} = \frac{d(\theta_1 + \theta_2 + \theta_3)Q}{dt} = \frac{d\theta_1 Q}{dt} + \frac{d\theta_2 Q}{dt} + \frac{d\theta_3 Q}{dt}$$

$$= \lambda(\theta_1 Q) - \lambda(\theta_2 Q) - \lambda(\theta_3 Q)$$

$$= \lambda Q(\theta_1 - \theta_2 - \theta_3)$$

$$= \lambda Q(2\theta_1 - \theta_1 - \theta_2 - \theta_3)$$

$$= \lambda Q(2\theta_1 - 1) \quad \text{as} \quad \theta_1 + \theta_2 + \theta_3 = 1$$

$$= \lambda \alpha Q \qquad \text{where} \quad \alpha = (2\theta_1 - 1)$$

$$\therefore Q_t = e^{\lambda \alpha t} Q_o$$

Finally, because every $\theta_2 Q$ division leads to a loss of one cell from the $\theta_2 Q$ population and a gain of 2 cells to the R population, the R population increases at twice the rate at which the $\theta_2 Q$ population diminishes.

$$\therefore \frac{dR}{dt} = -2\frac{d(\theta_2 Q)}{dt}$$

$$= 2\lambda(\theta_2 Q)$$

$$= \lambda 2\theta_2 Q$$

$$= \lambda\beta Q \qquad \text{where } \beta = 2\theta_2$$

$$\therefore Rt = \frac{\beta}{\alpha}(e^{\lambda\alpha t} - 1)\,Qo + Ro$$

Consequently

$$Nt = Pt + Qt + Rt$$

$$= e^{\lambda t}Po + e^{\lambda\alpha t}Qo + \left[\frac{\beta}{\alpha}(e^{\lambda\alpha t} - 1)Qo + Ro\right]$$

$$= e^{\lambda t}Po + (1 + \frac{\beta}{\alpha})Qo e^{\lambda\alpha t} - \frac{\beta}{\alpha}Qo + Ro$$

By considering the first and second derivatives of N_t we may deduce that N_t is an increasing function of t and may have a point of inflexion if:

$\alpha < o$, i.e. the store of Q cells is being diminished

$|\alpha| < \beta$ i.e. $\theta_1 + \theta_2 > \frac{1}{2}$ (Billewicz - personal communication)

and $\left|(1 + \frac{\beta}{\alpha})\alpha^2 Q_o\right| > P_o$

Model II

In Model II the initial assumptions are the same as for Model I but now cells of type R may attempt to divide but produce no viable cells. This is equivalent to assuming for R cells a mortality constant say γ. Thus we have now for R cells (Billewicz - personal communication):

$$\frac{dR}{dt} = \lambda\beta Q - \gamma R \text{ and } R = \frac{\lambda\beta}{\alpha\lambda + \gamma} Q_0 e^{\alpha\lambda t} + (R_0 - \frac{\lambda\beta}{\alpha\lambda + \gamma}Q_0)e^{-\gamma t}$$

giving $N_t = P_0 e^{\lambda t} + Q_0 e^{\alpha\lambda t} + \frac{\lambda\beta}{\alpha\lambda+\gamma} Q_0 e^{\alpha\lambda t} + (R_0 - \frac{\lambda\beta}{\alpha\lambda+\gamma} Q_0) e^{-\gamma t}$

In this case N_t is not necessarily an increasing function of t and may have a number of peculiar points depending on the choice of parameters.

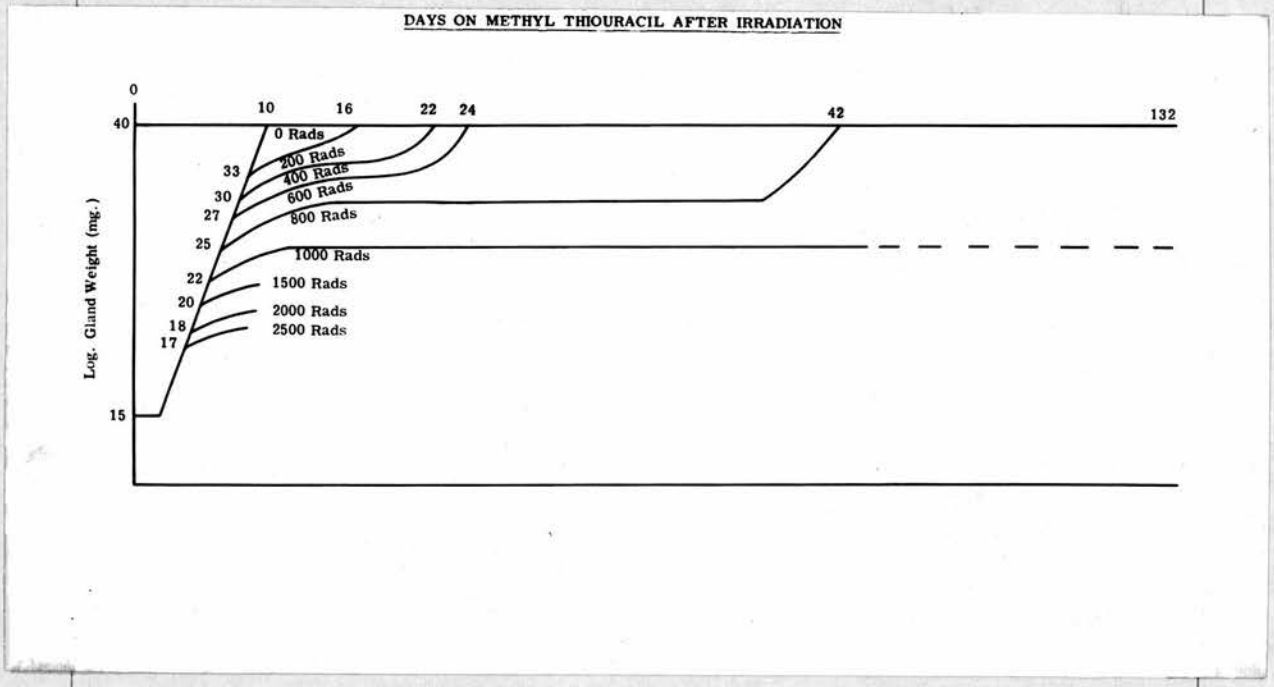
Unfortunately neither of these models can be fully determined. The constants β, Q_0 and R_0 cannot be estimated separately although $Q_0 + R_0$ can be estimated. Thus, as might have been anticipated it is impossible to deduce too much about the internal dynamics of a system from the behaviour of one aggregate measure (e.g. total follicular cell population) over a period of time. For example curves not dissimilar from those found in experiments G, H and J could be obtained by assuming that Q cells damaged by radiation can produce other Q cells only for a limited number of cycles and that their division constant (λ) differs from that for the undamaged cells.

In these circumstances it is necessary to look for a biologically acceptable model which fits the data well. In this case the growth curves are neatly and acceptably explained by the observations of Elkind et al. (1963).

Fig. 12

Diagrammatic Summary of Experiments G, H and J showing the effects of doses of x-rays in the range 200 to 2,500 rads on the goitrogenic response of the rat thyroid to methylthiouracil administration. The log mean gland weight is plotted against time on methylthiouracil after irradiation.

Fig. 12



Diagrammatic Summary of Experiments G, H and J showing the effects of doses of x-rays in the range 200 to 2,500 rads on the goitrogenic response of the rat thyroid to methylthiouracil administration. The log mean gland weight is plotted against time on methylthiouracil after irradiation.

DISCUSSION

Figure 12 summarises in diagrammatic fashion Experiments G, H and J in which the effect of doses of x-irradiation in the range 0 - 2,500 rads on the goitrogenic response of the rat thyroid were observed. The log of the increase in gland weight is plotted against the time after radiation on methylthiouracil. With no irradiation after a lag of 2 days the gland weight increases exponentially until a plateau is reached after approximately 10 - 12 days. In the radiated group it can be seen that the exponential growth is interrupted by a pause during which there is little or no increase in gland weight. The pause is terminated by a final growth spurt to the plateau weight. It can be seen that the pause occurs earlier and lasts longer with increasing doses of x-rays. With doses of between 1,500 and 2,500 rads many of the rats did not live longer than 14 - 20 days so that the duration of pause could not be observed. There is however a strong suggestion of the same lag phase, initial exponential growth and pause found with lower doses.

Estimates of the follicular cells in Experiments G, H and J showed that these remain remarkably constant irrespective of irradiation dose, time after irradiation, and time on methyl-thiouracil administration. It can be concluded therefore that the pattern for changes in gland weight parallels the changes in the total thyroid follicular cell populations.

The/

Fig. 13

Diagram to illustrate the hypothetical effect of x-radiation on the goitrogenic response assuming significant immediate cell death.

Fig. 13.

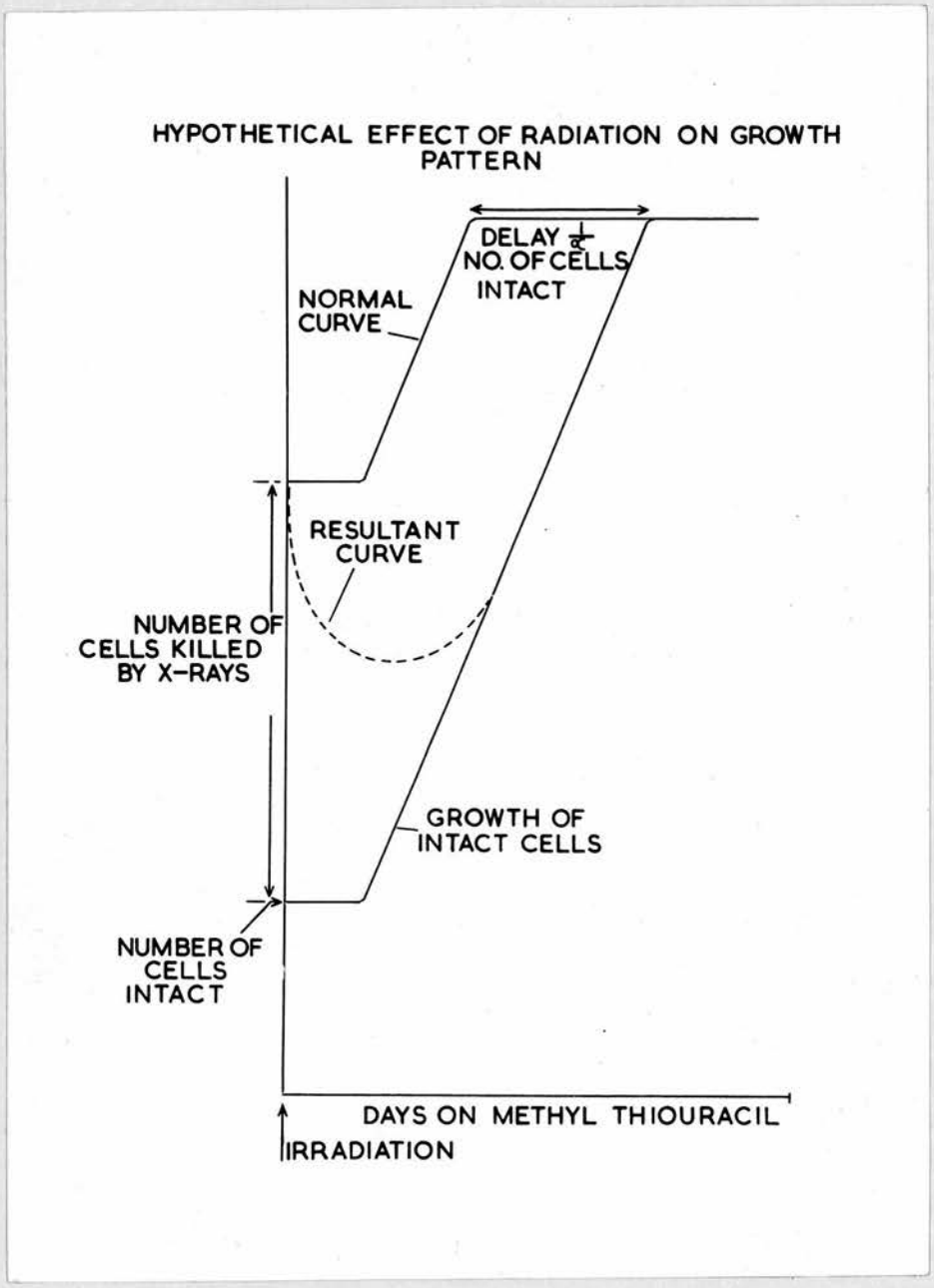


Diagram to illustrate the hypothetical effect of x-radiation on the gpitrogenic response assuming significant immediate cell death.

The pattern of the effects of the increasing doses of x-radiation on the goitrogenic response is strikingly similar to that obtained by Elkind et al. (1963) with cells in tissue culture. The following explanation of these patterns is put forward as being radiobiologically acceptable.

Firstly there is no evidence of significant immediate cell death. If this had been the case the hypothetical curve shown in Fig. 13 would have resulted. In addition there was no evidence of cell death during the phase of exponential cell division following methylthiouracil administration. Had significant "mitotic death" occurred, a phase of exponential growth in the irradiated glands would have been most unlikely and certainly exponential growth identical to that seen in the unirradiated controls impossible. It could be suggested that the pause in exponential growth was the resultant of cellular "mitotic death" and successful cell division on the part of reproductively intact cells. However no histological evidence of cell death was obtained either by visual assessment or by estimates of the number of follicular cells per unit volume and in addition there is a more attractive radiobiological explanation which finds a parallel in in vitro studies of Elkind et al. (1963).

Immediately after irradiation there exists a certain proportion of reproductively intact cells. This will decrease as the x-ray dose increases. By definition, these reproductively intact/

intact cells, will divide at the same rate as the unirradiated controls and can be held accountable for the growth spurt to the plateau weight which brings an end to the pause in growth. With higher doses there are very few intact cells at time zero after irradiation - only 2% (approximately) after 800 rads. Clearly successful division on the part of these cells can make no significant contribution to the increase in gland weight and follicular cell population for the first 10 days on methylthiouracil as according to the normal goitrogenic response there can be no more than 3 complete division cycles during this period (minimum division time = 4 days). The initial increase in the thyroid follicular cell population can therefore only be brought about by limited division on the part of damaged cells of the type described by Elkind et al. (1963). These cells divide at the same rate as the unirradiated controls but are capable only of limited division, the mean number of divisions being inversely related to the radiation dose. The pause in the exponential growth can be accounted for by exhaustion of the division potential of these damaged cells. As stated above the growth spurt which determinated this pause can best be accounted for by the presence of reproductively intact cells which finally "catch up" and carry the follicular cell population up to the plateau level attained by the unirradiated controls (see Fig. 13).

As/

As far as could be determined the slowly growing clones of cells of the type described by Sinclair (1964) made no significant contribution to growth particularly at the higher doses.

The main conclusions of these experiments are therefore as follows:-

(1) No immediate cell death with doses of x-rays up to 2,500 rads.

(2) The reproductively damaged cells start to divide after the normal lag phase of 2 days. Any lag imposed by irradiation (Elkind et al. 1963) must be buried within this "physiological" lag phase.

(3) The initial growth of the damaged cells takes place at a rate similar to that of the unirradiated controls.

(4) With increasing doses of x-rays fewer damaged cells divide fewer times.

(5) Doses which leave very few cells reproductively intact do not cause any significant death either in the short or long term. The corollary to this is that doses of radiation which do cause significant cell death will leave only an infinitely small proportion of cells reproductively intact. This is a point of great clinical importance which will shortly be discussed in greater detail.

(6) The striking parallel between these in vivo results and those of Elkind et al. (1963) in vitro are yet another demonstration of/

of the universality of radiobiological phenomena. This is also demonstrated by the similarity of the dose-response curve for follicular cell reproductive integrity to the Puck-Hewitt dose-response curve (Fig. 11). Both these observations suggest that the radiobiological phenomena involved are so fundamental as to be applicable to any type of mammalian cells provided they be well oxygenated and strengthen the case for extrapolation to human radiotherapeutics.

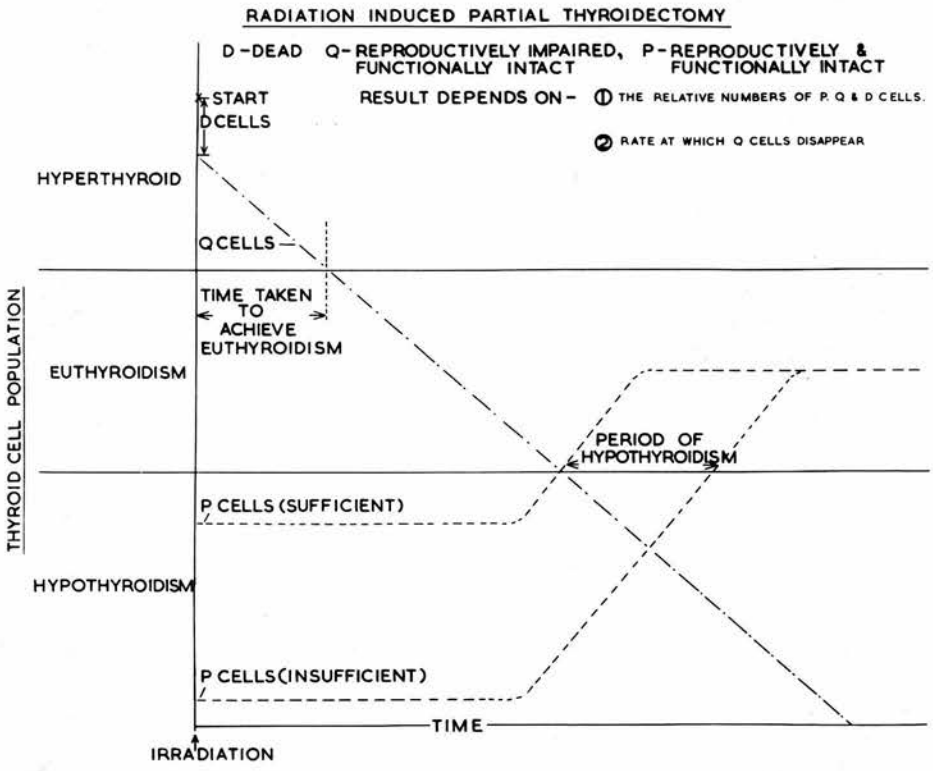
It should be noted at this point that doses of x-rays up to 1,000 rads have no long term effect on total thyroid function. Evidence for this will be provided in a subsequent section of the thesis. Consequently it can be taken that any decrease in the total thyroid function must result from cell death and that any residual function is proportional to the number of cells left alive albeit reproductively damaged. These concepts are relevant to the question of whether a radiation induced "partial thyroidectomy" can be brought about by homogeneous thyroidal irradiation from an external source (e.g. ^{60}Co.) and are brought together in diagrammatic form in Fig. 14. If we consider that a patient is hyperthyroid because he has too many follicular cells working "too hard" then we can render him euthyroid by killing off the correct number of cells and hypothyroid by killing too many. A dose of x-radiation can create 4 broad categories of cells.

(1)/

Fig. 14

Diagrammatic representation of the factors determining the feasibility of a radiation partial thyroidectomy. See text for further explanation of diagram.

Fig. 14



Diagrammatic representation of the factors determining the feasibility of a radiation partial thyroidectomy. See text for further explanation of Diagram.

(1) Dead Cells - D

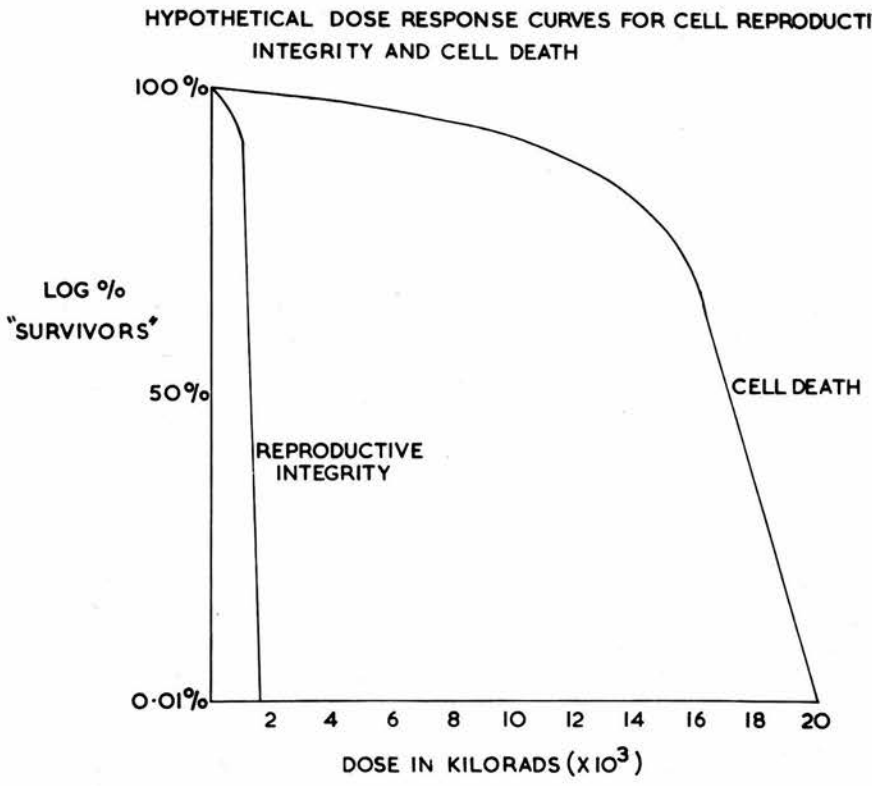
(2) Reproductively damaged but functionally intact - Q

(3) Both reproductively and functionally intact - P

(4) Cells which have lost their function as a result of x-irradiation but are still alive probably occur but for practical purposes can be discounted. Justification for this will be found in Section III of this thesis.

The result of irradiation for the patient will depend on the relative number of P, Q and D cells and the rate at which the Q cells disappear. Successful treatment can only be achieved if a dose of radiation can be found which will create a population of D and Q cells which die off quickly enough to make the patient euthyroid within a clinically acceptable period. At the same time sufficient P cells must be left so that as thyroid failure threatens as a result of the removal of Q cells the follicular cell population can be repleted. It has already been pointed out however that according to Experiments G, H and J, doses which leave very few cells reproductively intact (P) do not cause any significant cell death (D). The corollary to this is that doses which do cause significant cell death can leave only an infinitely small proportion of cells reproductively intact. In other words there must be a wide separation between the dose response curve for cell reproductive integrity and that for cell death as shown by the hypothetical/

Fig. 15



Diagrammatic representation of the divergence between the relative radiosensitivities of cell reproductive integrity and cell death.

hypothetical dose response curves in Fig. 15. Thus the results strongly suggest that if the first condition is satisfied - namely the creation of a significant proportion of D and Q cells then insufficient P cells will be left so that hypothyroidism will inevitably come about. Theoretically if the small number of P cells were left for long enough under endogenous TSH stimulation they might replete the thyroid follicular cell population. This would be ethically unjustifiable however, and institution of thyroxine replacement therapy when hypothyroidism threatens removes endogenous TSH stimulation and ensures that cell repletion can never occur.

It is concluded therefore that it is intrinsically impossible to successfully treat thyrotoxicosis with the homogeneous organ irradiation obtained from an external source because it is impossible to destroy part of the thyroid gland without rendering the remaining cells incapable of further division. In other words a "radiation partial thyroidectomy" which requires the combination of significant cell death and significant cell reproductive survival cannot be attained. This awkward radiobiological fact can be got around by using radioiodine (^{131}I), which is concentrated by the thyroid, to deliver the dose of radiation. Because of the patchy distribution of this isotope throughout the gland "hot spots" occur where massive doses of radiation sufficient to effect cell death can be attained and/

and at the same time leave intervening areas undamaged. Unfortunately this patchy distribution is unpredictable and is seldom so marked that significant areas of the thyroid gland are totally spared from radiation damage. It could therefore be predicted that while radioiodine therapy would obviate, to some extent, the intrinsic impossibility of a "radiation partial thyroidectomy" with homogeneous irradiation, the results of therapy would be extremely variable. These predictions find confirmation in the clinical trials of external radiation and low doses of radioiodine (^{131}I) reported in Section IV.

In this discussion one important factor remains to be accounted for, namely the rate of cell death as a result of radiation damage. It has been suggested that hypothyroidism comes about in radioiodine treated patients as a result of cell fall-out with failure of cell replacement because of loss of reproductive integrity on the part of the remaining cells. This concept finds support from the results of Al-Hindawi and Wilson (1965) who demonstrated accelerated cell loss in the radioiodine treated rat thyroid. As already mentioned, there was no evidence of significant cell death over a period of 1 year after 1,000 rads x-irradiation in the experiments reported here and it is probable that the method employed was not sufficiently sensitive. Indirect evidence for cell death has been obtained by Weir (1967) using immunological techniques. It is hoped to obtain evidence/

evidence of cell death in irradiated rat thyroid by this means and the results will be reported elsewhere. Finally, however, the significant hypothyroid rate (5 to 30%) in thyrotoxic patients treated by partial thyroidectomy must be taken into account (Hershman, 1966). This strongly suggests that the hypothyroid rate following ^{131}I therapy cannot be entirely accounted for by radiation damage and that some other pathological process is operating.

SUMMARY

The effects of x-irradiation in the range 0 to 2,500 rads on the goitrogenic response of the rat thyroid to methylthiouracil were studied. It was found that the number of follicular cells per unit volume remained constant irrespective of dose of radiation, time after radiation and time on methylthiouracil. Consequently the changes in thyroid gland weight could be equated with changes in the total follicular cell population of the thyroid.

The behaviour of the mean total follicular cell population followed a clear pattern in response to the graded doses of x-rays. The results suggested that in the dose range employed no immediate cell death occurred. Furthermore, the first division cycle of intact and damaged cells proceeded at the same rate with no significant mitotic death occurring in the latter. With increasing doses of x-rays fewer cells divided fewer times. The fact the doses of x-rays (e.g. 1,000 rads) which left very few cells reproductively intact caused no significant cell death either in the short or long-term indicated that doses of radiation which do cause significant cell death will leave only an infinitely small proportion of cells reproductively intact. This means that a "radiation partial thyroidectomy" with homogeneous (e.g. external) radiation is intrinsically impossible in the rat.

The similarity of the observed dose/response characteristics to/

to those obtained with other mammalian cells *in vivo* and *in vitro* suggests that the radiobiological phenomena underlying these observations are common to all mammalian cells irrespective of tissue or species of origin. These facts provide considerable justification for extrapolation of the above conclusions to the radiotherapeutics of thyrotoxicosis in man.

SECTION III

The Long and Short Term Effects of X-radiation on the Iodide Trapping Function of the Rat Thyroid.

INTRODUCTION

In Section II the effects of x-radiation on follicular cell viability and reproductive capacity were studied. From the clinical point of view, however, it is not these parameters which are important but the effect of radiation on net thyroid function. In other words, the aim of treatment of hyperthyroidism with ionising radiation is to permanently reduce net thyroid function to normal levels. Theoretically this could be achieved by follicular cell death (immediate or delayed) or by the induction of significant numbers of follicular cell mutants which, though viable, were incapable of synthesising thyroid hormone.

The effects of radioiodine (^{131}I) on thyroid function in the rat have been studied by Feller, Chaikoff, Taurog & Jones (1949), Maloof, Dobyns and Vickery (1952) and Doniach and Logothetopoulos (1955). With "estimated" rad doses of the order of 10,000 rads these workers found little effect on net thyroid function although as discussed in Section II there was considerable diminution of reproductive capacity of the follicular cell population. These results are consistent with those of Crooks et al. (1964) who found that accurately measured doses of x-rays up to 1,600 rads had no effect on the iodide trapping ability of the rat thyroid at 1 and 4 weeks after irradiation.

As/

As an extension of the studies of Crooks et al. (1964) it was decided to study the effects of 1,000 rads x-rays at intervals up to one year after irradiation and the short term effects of x-ray doses in the range 500 to 2,500 rads during the first 96 hours after irradiation. Long term studies of the effects of doses greater than 1,000 rads were not carried out because of the high mortality associated with doses of this order (see Section II). It was known from the studies reported in Section II that with these doses of x-rays there was no evidence of significant cell death during the periods of observation after irradiation. Any changes in overall thyroid function would therefore have to be explained by other radiobiologically acceptable mechanisms.

MATERIALS AND METHODS

Long Term Effects of X-radiation on Iodide Trapping

Adult, male Wistar rats were randomly constituted into 10 groups of 5 animals each. All the animals received 1,000 rads x-radiation to their thyroids by the technique described in Section II. At intervals of 0, 1, 3, 7, 14 and 22 days and 3, 6, 9 and 12 months after initial radiation one group of animals was given an intraperitoneal injection of 0.5 μCi ^{132}I. One hour later the animals were killed by prolonged chloroform anaesthesia, the thyroid glands removed and digested for 24 hours in 3N. NaOH. The ^{132}I content of these digests were then measured by radioactive counting in an automatic well-type scintillation counter (Gammamatic: Nuclear Enterprises Ltd.), corrected for radioactive decay and expressed as a percentage of the original (0.5 μCi) dose.

Short Term Effects of X-radiation on Iodide Trapping

Adult, male Wistar rats were randomly constituted in 32 groups of 5 animals each. Two groups received no radiation and were used to determine the control 1 hour percentage uptake of ^{132}I at the beginning (0 hours) and end (96th hour) of the experiment. Six groups each were treated with 500 rads, 1,000 rads, 1,500 rads, 2,000 rads and 2,500 rads x-radiation to their thyroid glands as described in Section II. At intervals of 0, 5, 23, 47 and 95 hours after/

after irradiation one group of animals from each radiation dose level was given an intraperitoneal injection of 0.5 μCi ^{132}I and the 1 hour percentage uptake of the isotope determined as above.

TABLE VIII

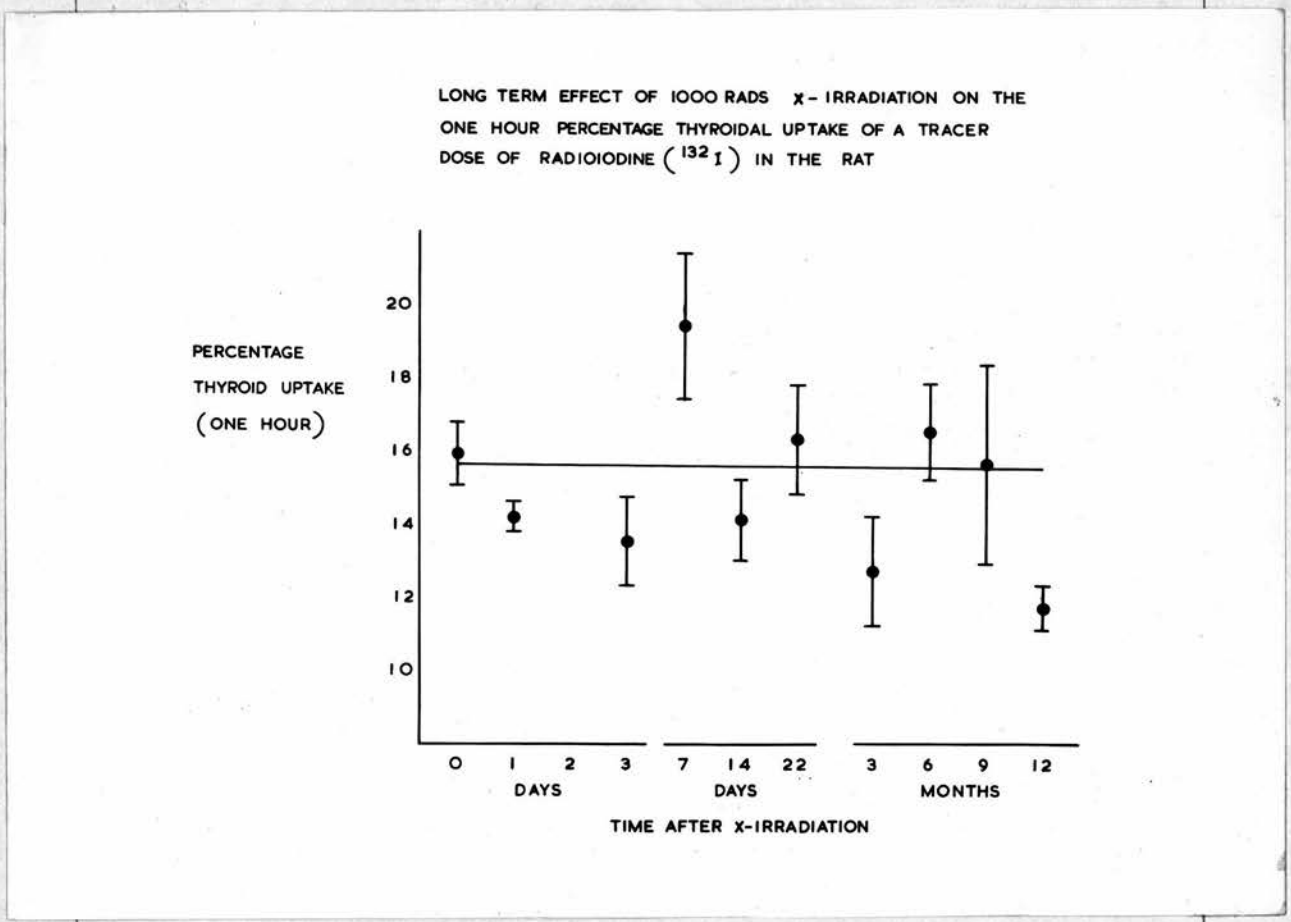
Long-Term Effect of 1,000 rads X-Radiation on the One Hour Percentage Thyroidal Uptake of a Tracer Dose of Radioiodine (^{132}I) in the Rat

Time After Irradiation		% Thyroid Uptake (1 hr.) of a Tracer Dose of ^{132}I
0		15.9±0.8
1 day		14.2±0.4
3 days		13.5±1.2
7 days		19.4±2.0
14 days		14.1±1.1
22 days		16.3±1.5
3 months		12.7±1.5
6 months		16.5±1.3
9 months		15.6±2.7
12 months		11.7±0.6
Regression Equation $Y = a + bx$		$Y = 15.64 - 0.01X$
Analysis of Variance of Regression	F Ratio for $b \neq 0$	2.5 ($p > 0.05$)
	F Ratio for Deviation From Rectilinearity	< 1 ($p > 0.05$)

Fig. 16

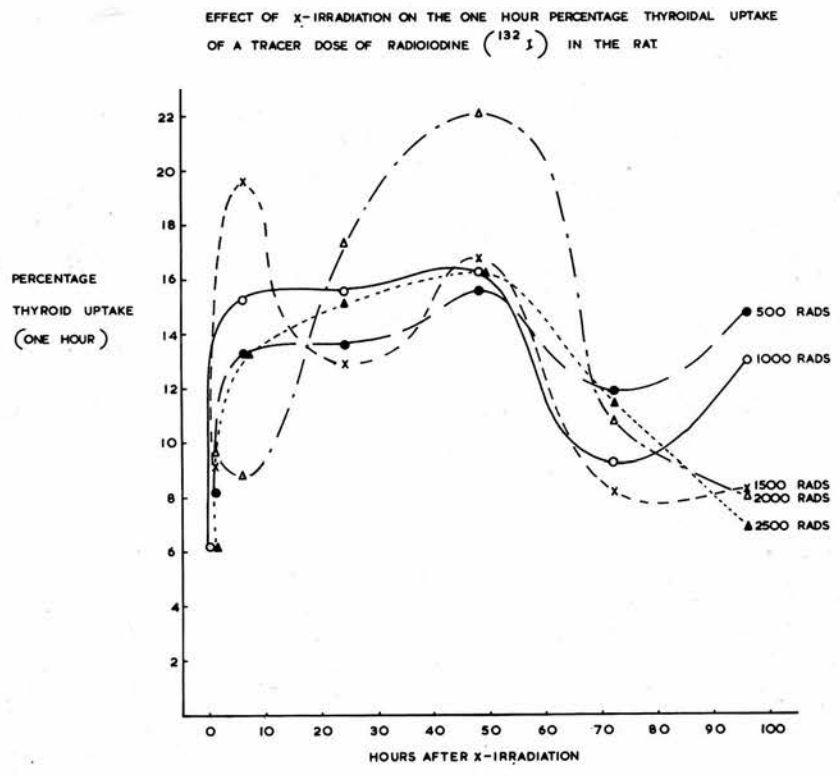
The long term effect of 1,000 rads x-irradiation on the one hour percentage thyroidal uptake of a tracer dose of radioiodine (^{132}I) in the rat. The mean one hour percentage uptake is plotted against time after irradiation.

Fig. 16



The long term effect of 1,000 rads x-irradiation on the one hour percentage thyroidal uptake of a tracer dose of radioiodine (^{132}I) in the rat. The mean one hour percentage uptake is plotted against time after irradiation.

Fig. 17



The short term effects of doses of x-radiation in the range 500 to 2,500 rads on the one hour percentage uptake of ^{132}I by the rat thyroid. The one hour percentage uptake is plotted against hours after irradiation.
See Table IX for standard errors.

RESULTS

Long Term Effects of X-radiation on Iodide Trapping

The mean 1 hour percentage uptakes (± standard error (S.E.)) of the tracer dose of ^{132}I at intervals up to one year after 1,000 rads x-irradiation are contained in Table VIII (Appendix Table 20) and shown diagrammatically in Fig. 16. Analysis of variance of these results showed that the regression equation of percentage radio-iodine uptake on time after irradiation ($Y = 15.64 - 0.01X$) showed no significant deviation from rectilinearity and no significant deviation from zero slope ($p > 0.05$). Thus 1,000 rads x-radiation had no long term effect on the iodide trapping function of the rat thyroid.

Short Term Effects of X-radiation on Iodide Trapping

The mean one hour percentage uptakes (± S.E.) of the tracer dose of ^{132}I at intervals up to 96 hours after irradiation are contained in Table IX (Appendix Table 21) and shown diagrammatically in Fig. 17. Analysis of variance was performed on these data and revealed that, with the exception of the 500 rad group, at all other levels (1,000, 1,500, 2,000 and 2,500 rads) there was significant deviation from rectilinearity of the regression of one hour radio-iodine uptake on time up to 96 hours after irradiation ($p < 0.01$). Thus doses of x-rays of this order altered iodide trapping during this period.

In/

TABLE IX

Short-Term Effects of X-Irradiation on the One Hour Percentage Thyroidal Uptake of a Tracer Dose of Radioiodine (^{132}I) in the Rat

Hours After Irradiation		Mean Thyroid 60 Minute Uptake of Radioiodine (%)					
		0 rads	500 rads	1,000 rads	1,500 rads	2,000 rads	2,500 rads
0		12.7±1.2					
1		-	8.2±0.9	6.2±0.6	9.2±1.5	9.7±0.7	6.2±0.6
6		-	13.3±1.4	15.3±2.4	19.6±1.9	8.8±1.0	13.3±1.4
24		-	13.6±1.5	15.6±1.1	12.9±1.4	17.4±1.9	15.2±2.0
48		-	15.6±2.2	16.3±1.8	16.8±1.2	22.1±1.6	16.3±1.0
72		-	11.9±0.9	9.3±1.2	8.2±1.0	10.8±0.6	11.5±1.3
96		11.3±0.7	14.8±1.8	13.0±1.9	8.3±1.2	8.1±0.9	6.9±0.8
Regression Equation $Y = a + bx$			$Y = 11.53+0.03x$	$Y = 12.47+0.00x$	$Y = 15.03-0.07x$	$Y = 13.93-0.02x$	$Y = 12.29-0.02x$
Analysis of Variance of Regression	F Ratio for $b \neq 0$		< 1	< 1	$10.2 (p<0.01)$	< 1	< 1
	F Ratio for Deviation From Rectilinearity		< 1	$4.0 (p<0.01)$	$14.2 (p<0.01)$	$85.3 (p<0.01)$	$4.4 (p<0.01)$

In addition, at each radiation dose level employed, there was consistent depression of radioiodine uptake immediately (i.e. during the first hour after irradiation with complete recovery by 6 to 24 hours after irradiation. Inspection of the curves also suggested a second phase of decreased uptake at 76 hours after irradiation which persists to 96 hours in the case of the 1,500, 2,000 and 2,500 rad groups.

DISCUSSION

The absence of any diminution of iodide trapping by the rat thyroid over the year following 1,000 rads x-irradiation of the gland is consistent with the findings of previous investigators (Feller, 1949; Maloof et al. 1952; Doniach and Logothetopoulos, 1955; Crooks et al. 1964) and support their view that doses of radiation which profoundly impair the reproductive capacity of the rat thyroid follicular cell population have no significant effect on net thyroid function. It has been shown in Section II that with this dose of x-radiation there is no significant cell death up to one year after irradiation. It can therefore be concluded that 1,000 rads x-radiation does not produce a significant number of viable follicular cell mutants which have lost their ability to synthesise thyroid hormone. In this regard, some calculations based on data published by Puck (1960) are of interest. It is not claimed that these calculations represent the total radiobiological phenomena underlying the observed effects. However, the conclusions deduced from Puck's original observations appear to have general validity.

In radiation studies with euploid mammalian cells in tissue culture Puck (1960) found that with doses of 600 rads (i.e. approximately 6 D_{37} doses for reproductive integrity) at least 80% of the cells retaining reproductive integrity showed chromosomal damage/

damage of some kind, e.g. morphological change or observable nutritional mutations. Now, in radiobiological target theory an 80% effect of any kind is brought about by 1.6 D_{37} doses. Therefore the D_{37} dose for damage to those parts of the chromosomes not essential for cell division is $\frac{600}{1.6}$ = 400 rads. Assuming equal susceptibility of the target material the D_{37} dose for any particular characteristic is inversely proportional to target size, i.e. the smaller the target the larger the quantity of radiation needed to have, say, a 63% chance of inactivating it.

Therefore the amount of genetic material concerned with cell division is greater than that concerned with all other independent functions in the inverse ratio of their D_{37} doses = $\frac{400}{100} = \frac{4}{1}$.

Therefore only $\frac{1}{4}$ of the total genetic mass is not concerned in some way with cell division and its D_{37} dose is 400 rads.

But, it has been estimated that there are approximately 1,000 different enzymes in mammalian cells (Harper, 1959). Therefore assuming proportional representation of these enzymes in the "genetic parliament" $\frac{1}{1000}$ part of the total genetic constitution controls each enzyme. As there are probably five enzymes concerned with thyroxine synthesis these will be controlled by $\frac{5}{1000} = \frac{1}{200}$ part of the total genetic mass.

But if $\frac{1}{4}$ of the total genetic mass has a D_{37} dose of 400 rads, $\frac{1}{200}$ part of the total genetic mass will have a proportionately much greater/

greater D_{37} dose

$$\text{i.e.} \quad \frac{200}{1} \times \frac{400}{4} = \underline{20{,}000 \text{ rads}}$$

Thus, in a thyrotoxic patient 20,000 rads would reduce thyroid function (i.e. thyroid hormone production) to 37% of its previous level by inactivation of the genes responsible for thyroid hormone synthesis. Assuming that this dose is in fact given and remembering that the D_{37} dose for cell division is of the order of 100 rads the high incidence of myxoedema following radioiodine therapy is immediately understandable. As 20,000 rads = 200 D_{37} doses for cell division the surviving fraction of reproductively intact cells will be $(\frac{1}{e})^{200} = 10^{-87}$. It is interesting that assuming 10^6 follicular cells per mm^3 (see Section II) and a tissue specific gravity of unity, 10^{87} human thyroid cells would represent a mass of 10^{75} Kg. (mass of the earth = 10^{24} Kg)!

Conversely if 100 rads are delivered, 67% of the thyroid cell population will lose their reproductive integrity. However 100 rads is only $\frac{1}{200}$ of the D_{37} dose for thyroxine production. Consequently the diminution in thyroid function will only be $(\frac{1}{e})^{\frac{1}{200}} = < 1\%$.

Thus, the so-called "dissociation" in radiosensitivity of thyroid follicular cell function and reproductive integrity (Crooks et al. 1964) is merely a reflection of the relative radiation target/

target sizes offered by the genetic masses relevant to these two aspects of cell activity. The D_{37} dose for cell reproductive integrity appears to be in the range 100 to 200 rads (Puck et al. 1957; Hewitt and Wilson, 1961), that for cell death of the order to 10,000 rads (St. Aubin et al. 1957) and that for thyroid hormone synthesis around 20,000 rads (vide supra). These wide differences in the D_{37} doses for cell division, viability and function offer an attractive explanation, at the molecular level, of the conclusion reached in Section II that a radiation partial thyroidectomy with homogeneous organ irradiation is intrinsically impossible. In other words, for clinical purposes, a reduction in thyroid function can only be achieved by bringing about significant cell death. However, doses of radiation high enough for this will leave an infinitely small number of cells reproductively intact. As the remaining viable (but non-reproducable) cells probably possess a shortened life span (Al-Hindawi and Wilson, 1965) thyroid failure will inevitably supervene due to accelerated cell death and failure of adequate cell replacement. For practical purposes viable clones of non-functioning cells can be regarded as making no significant contribution to decreased thyroid activity because the D_{37} dose for cell death is probably very much lower than that for cell function.

Short Term Effects of X-radiation on Iodide Trapping Function

The effects of doses of x-radiation in the range 500 to 2,500 rads/

rads during the first 96 hours after irradiation contrast with the complete absence of effects in the long term. The biphasic depression of iodide trapping is reminiscent of similar effects found by Trotter and Willoughby (1967) in thyrotoxic patients treated with 400 to 1,000 rads γ-radiation (^{60}Co source) to their thyroids, although the time relations are different. These workers suggested that arterial constriction or capillary compression by oedema following radiation resulted in diminished thyroid perfusion and a consequent fall in iodide trapping. This view finds support in the observation of arterial vasoconstriction during the 24 hours following irradiation of the hind leg in rabbits (Moss and Gold, 1963) and also from the fact that the iodide trapping capacity of either thyroid lobe in humans can be decreased by irradiating the upper pole of the lobe only (Trotter and Willoughby, 1967). Whatever the explanation for these phenomena, it is clear, in view of the absence of a long term effect in the rat, that the processes are reversible. In view of this, another possible explanation is damage to enzymes in the cell membrane and cytoplasm which provide a large radiobiological target. Damage of this type could presumably be repaired providing the genes responsible for their synthesis are left intact and would provide an attractive explanation of the depression of iodide trapping in the first hour after irradiation, with enzyme resynthesis responsible for the recovery/

recovery of function during the following 24 hours.

It is difficult to conceive an easy explanation of the depression of iodide trapping which occurred at 72 hours after irradiation in these experiments. Although Moss and Gold (1963) observed a second phase of vasoconstriction in the irradiated leg of rabbits and Trotter and Willoughby (1967) a second phase of depression of iodide trapping in man, these occurred at 4 to 6 weeks and 3 weeks respectively after initial radiation. Clearly further investigations are required to find an explanation of these phenomena.

SUMMARY

Studies of the effect of 1,000 rads xOradiation on the iodide trapping capacity of the rat thyroid at intervals up to one year after irradiation revealed no impairment of thyroid function. This observation is consistent with those of previous workers (Feller et al. 1949; Doniach and Logothetopoulos, 1955; Crooks et al. 1964). Previous studies (see Section II) had shown that 1,000 rads x-radiation did not cause significant cell death during this period. Consequently it can be concluded that x-ray doses of this order do not produce significant numbers of follicular cell mutants which are incapable of synthesising thyroid hormone.

The fluctuations in iodide trapping by the thyroid during the first 96 hours after x-irradiation in the range 500 to 2,500 rads are in some respects similar to those found by Trotter and Willoughby (1967) in thyrotoxic patients and are of considerable radiobiological interest. Arterial damage and capillary compression from oedema resulting in tissue ischaemia and cytoplasmic enzyme damage are possible explanations for these phenomena which require further investigation.

SECTION IV

The Treatment of Thyrotoxicosis with Low Doses of Ionising Radiation

INTRODUCTION

Radioiodine (^{131}I) therapy for thyrotoxicosis is not ideal. The disadvantages have been reported by Beling and Einhorn (1961), Dunn and Chapman (1964), Green and Wilson (1964), McGirr, Thompson and Murray (1964), Crooks (1965) and Nofal, Beierwaltes and Patno (1966). In the series reported by Crooks (1965), 40% of patients were still thyrotoxic eight months after initial ^{131}I therapy and 40% had become hypothyroid eight years after therapy with no evidence of a plateau in the cumulative incidence of thyroid failure. Thus we have the short term disadvantages of slow and unpredictable control of symptoms for the individual patient and the long term disadvantages of hypothyroidism developing many years after initial therapy. Smith and Wilson (1967) reported a trial of a low dose ^{131}I regime which attempted to overcome both these disadvantages. They gave "calculated rad doses" of 3,500 rads in contrast to orthodox levels of 7,000 to 10,000 rads. This procedure lowered the incidence of hypothyroidism at five years after therapy to 7.4% compared to 29% in the orthodox dose group. However, as a result of the lower dose employed there was an even greater delay in attaining the euthyroid state and antithyroid drugs were required in 64% of patients compared to 43% in the orthodox dose group. A similar trial using low doses of ^{131}I was carried out by Hagen Ouelette and Chapman (1967) with similar results.

In/

In an attempt to further diminish the incidence of hypothyroidism it was decided to investigate the effects of even lower doses of ^{131}I in the treatment of thyrotoxicosis. A "calculated rad dose" of 2,400 rads was arbitrarily selected for this study using the same method of ^{131}I millicurie dose prescription as that employed by Smith and Wilson (1967).

As successful x-ray therapy of thyrotoxicosis had been reported by several authors it was also decided to treat a group of patients with external radiation from a ^{60}Co source. Hayes (1927) stated that 62% of his cases were cured and 14% improved by x-irradiation of their thyroids and Groover, Christie, Merritt, Coe and McPeak (1929) claimed an 89% cure rate and 9% improved. Soley and Stone (1942) reported cure in 58% of patients and improvement in 18% following a total thyroid radiation dose of 900 roentgens in air given over six days in addition to 450 roentgens to the thymus over the same period. Unfortunately it is impossible to calculate the total rad dose in the other studies as full details of the x-ray machines and procedure are not provided. In view of the radiobiological studies of Puck, Morkovin, Marcus and Cieciura (1957) and Hewitt and Wilson (1961) it was thought that single doses in the range 100 to 1,000 rads might have a significant effect on thyroid function. This approach was made more acceptable by the fact that the patients' symptoms could be controlled with antithyroid drugs while/

while awaiting the definitive results of the radiation therapy. The use of antithyroid drugs offered an additional theoretical advantage. It had been demonstrated by Weinbren, Fitschen and Cohen (1960) that when irradiated rat liver cells were made to divide they underwent "mitotic death". It was hoped that the use of antithyroid drugs would not only control the patients' symptoms in a predictable way but by increasing the rate of thyroid cell turnover would hasten the attainment of a "radiation partial thyroidectomy" and that the amount of ultimate tissue destruction would be proportional to the dose of γ-rays delivered.

In all the reported studies of x-ray therapy of thyrotoxicosis the total dose of x-rays was fractionated over days or even weeks in order to diminish damage to the skin over the thyroid region. With γ-rays from ^{60}Co source large doses can be given without the danger of significant cutaneous scarring. Furthermore any dose of radiation is much more effective when given in one treatment than when it is fractionated (Ellis, 1963). For both these reasons it was hoped to improve on the results obtained by Soley and Stone (1942).

It must be emphasised that this study was carried out at the same time as the experiments reported in Sections II and III. Had the latter results been available beforehand, it is unlikely that the clinical trial of external radiation in the treatment of thyrotoxicosis would have been undertaken.

METHODS

Low Dose ^{131}I Therapy

Thirty thyrotoxic patients (23 women and 7 men) were treated, all of whom were over 40 years of age, had had no previous treatment for thyrotoxicosis and were not so ill that rapid control was required. The patients were assessed by means of general clinical examination, the Thyrotoxicosis Diagnostic Index shown in Table X (Crooks, Murray and Wayne, 1959), serum protein bound iodine (P.B.I.) estimation and standard radioiodine studies (Goodwin, Macgregor, Miller and Wayne, 1951). The weight of the thyroid in grammes was assessed from palpation of the gland by the same observer (M.T.H.) The therapeutic doses of ^{131}I required to deliver 2,400 rads were calculated from the formula shown which was derived from that of Blomfield, Eckert, Fisher, Miller, Munro and Wilson (1959).

$$\text{i.e. Dose of } ^{131}I \text{ (mCi)} = \frac{3 \times \text{mass of thyroid (g)}}{\text{48 hour uptake of } ^{131}I}$$

The doses were measured to the nearest 0.5 mCi. The mean dose was 2.8 ± 1.0 mCi (mean ± standard deviation). One patient with a large goitre and unusually rapid discharge of ^{131}I at 48 hours received 8 mCi.

The patients were reviewed at fortnightly intervals and clinical progress assessed by the Therapeutic Index shown in Table XI (Crooks, Robb and Wayne, 1960) and serum P.B.I. If there was evidence of improvement/

TABLE: X

Thyrotoxicosis - Diagnostic Index

Symptoms of recent onset and/or increased severity	Present	Absent	Signs	Present	Absent
Dyspnoea on effort	+1		Palpable thyroid	+3	-3
Palpitations	+2		Bruit over thyroid	+2	-2
Tiredness	+2		Exophthalmos	+2	L mm. R mm.
Preference for heat (irrespective of duration)		-5	Lid retraction Lid lag	+2 +1	
Preference for cold	+5		Hyperkinesis	+4	-2
Excessive sweating	+3		Finger tremor	+1	
Nervousness	+2		Hands hot	+2	-2
			moist	+1	-1
Appetite increased decreased	+3	-3	Casual pulse rate		min.
Weight		lbs.	Less than 80/min.		-3
increased		-3	More than 90/min.	+3	
decreased	+3		Aur. fibrillation	+4	
Totals			Totals		
Total symptom score			Total sign score		
	Total score			Toxic - greater than 19 Equivocal - 11-19 Euthyroid - less than 11	

TABLE: XI

Thyrotoxicosis - Therapeutic Index

Symptoms	Initially present and still present	Signs	
		Gland Size	cm.
Dyspnoea on effort	+1	Bruit over thyroid	Yes/No
Palpitations	+2	Exophthalmos	L mm
Tiredness	+2		R mm
Preference for cold	+5	Lid Retraction Lid lag	
Excessive sweating	+3	Conjunctival oedema	
Nervousness	+2		If present
Appetite increased	+1	Hyperkinesis	+4
Weight Decreased Increased Score (-1) for each increase of 4lbs.	lbs. +4	Hands hot moist	+2 +1
		Finger tremor	+1
		Casual pulse rate	/min.
		Greater than 85/min.	+3
Total symptom score		Total sign score	
	Total score		Normal range = 0-5 Thyrotoxic > 5

improvement at six weeks the observations were continued at monthly intervals until the patient was euthyroid. If no improvement was observed at 6 weeks, antithyroid drug therapy with carbimazole 15 mg. four times daily for two weeks and then 10 mg. thrice daily was given until the patient became euthyroid. Drug therapy was then stopped and the patient seen monthly. If thyrotoxicosis recurred the patient was treated with ^{131}I in a dose calculated to deliver 8,000 rads (Blomfield et al. 1959).

Radiation Therapy From a ^{60}Co Source

In this group, 28 thyrotoxic patients (21 women and 7 men) were accepted for the trial by the same criteria as used in the group receiving low doses of ^{131}I. The doses of γ-radiation were delivered from a ^{60}Co source (Orbitron; Allied Electrical Industries) under standard conditions. The day following radiation each patient was started on carbimazole 15 mg. four times daily for 2 weeks following which the dose was reduced to 10 mg. thrice daily until the patient was euthyroid whereupon the drug was stopped. During the period on carbimazole the patient's progress was assessed every 2 weeks and thereafter at less frequent intervals. Assessment at all stages was carried out as in the low dose ^{131}I study. If thyrotoxicosis recurred carbimazole therapy was restarted and the patient kept euthyroid for six months before stopping again.

The doses of γ-radiation are shown in Table XII and range from

115/

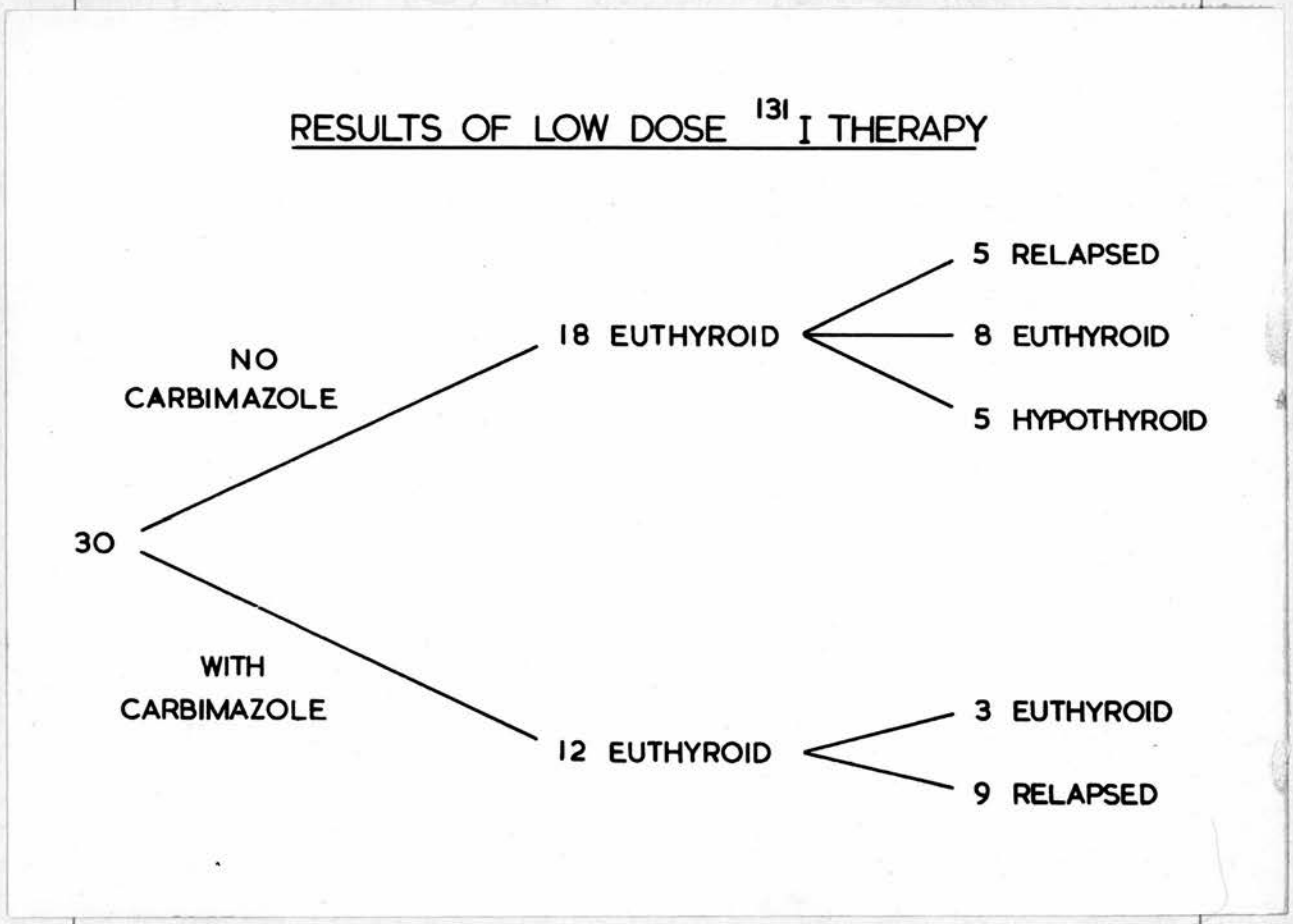
115 to 900 rads. Two patients received 115 rads, 4 patients 150 rads, 1 patient 180 rads, 7 patients 400 rads, 9 patients 600 rads, 1 patient 700 rads, 10 patients 800 rads and 7 patients 900 rads. In all, 28 patients, 13 of whom were treated twice, received 41 therapy doses of γ-radiation. All received carbimazole therapy for up to one year after therapy and all patients have been followed for 2 to 3 years from the time of initial therapy. One patient who relapsed after two therapeutic doses of 400 and 600 rads and another (E.C.) who relapsed $2\frac{1}{2}$ years after 180 rads were treated by partial thyroidectomy and the gross and histological features of their thyroids examined. All other relapses were treated with orthodox doses of ^{131}I (Macgregor, 1957).

In five patients the effect of 800 rads γ-radiation on thyroid function was measured by means of serial half-hour thyroid uptakes of tracer doses (20 μCi) of ^{132}I and the rate of discharge of a tracer dose (20 μCi) of ^{131}I before and after γ-radiation. The measurements of ^{132}I uptake in the presence of pre-existing uptake of ^{131}I was carried out by the method of Buchanan, Tulloch and Aboul-Khair (1963).

Fig. 18

Diagrammatic summary of the results of the clinical trial of low doses of radioiodine (^{131}I) in the treatment of thyrotoxicosis.

Fig. 18



Diagrammatic summary of the results of the clinical trial of low doses of radioiodine (^{131}I) in the treatment of thyrotoxicosis.

RESULTS

Low Dose ^{131}I Therapy

The results of therapy are shown in Fig. 18. In eighteen patients ^{131}I therapy alone produced the euthyroid state. The average time taken to become euthyroid was 8.9 weeks with a range of 4-18 weeks. Of these, 8 patients have remained euthyroid over a 2 to 3 year follow-up period. Five patients have relapsed and 5 patients have become hypothyroid. The length of the euthyroid period prior to relapse was 7-38 months and the interval between ^{131}I therapy and the development of hypothyroidism was 2-36 months. The patients who developed hypothyroidism were treated with thyroxine. In each of these patients thyroxine therapy was later stopped to assess whether thyroid function had recovered but all five were found to have permanent hypothyroidism. One patient developed transient hypothyroidism with an unrecordably low serum P.B.I. three months after radioiodine therapy but there was spontaneous recovery of thyroid function three months later and this patient has remained euthyroid over the subsequent three years.

Twelve patients showed little response to the ^{131}I therapy within six weeks and were therefore treated with carbimazole until euthyroid. The average duration of carbimazole therapy was 12.1 weeks and no patient received it for longer than 14 weeks. Of these patients, 8 relapsed as soon as carbimazole was discontinued and 1 relapsed after remaining euthyroid for 9 months after stopping carbimazole./

carbimazole. The other 3 patients have remained euthyroid for 2 to 3 years since cessation of carbimazole therapy.

No differences in the results of therapy were noted between those patients with diffuse goitres (14), those with nodular goitres (9) and those with goitres of doubtful consistency (8).

External Radiation Therapy

The results of external radiation therapy are shown in Table XII. All but three of the 28 patients had a recurrence of thyrotoxicosis within 8 months of stopping carbimazole therapy, the mean interval for relapse being 2.8 months. One of these three patients has remained euthyroid for three years after stopping carbimazole therapy. She has a normal serum P.B.I., a four-hour radioiodine uptake of 18% with significant suppression of uptake after 7 days treatment with tri-iodothyronine 100 µg. daily in 5 divided doses. The other two patients have remained clinically euthyroid for two years after stopping carbimazole, have normal serum P.B.I's. and four-hour radioiodine uptakes of 14% and 42% with no significant suppression of uptake with tri-iodothyronine administration.

Two patients were treated by partial thyroidectomy following recurrence of thyrotoxicosis. In one case (E.C.) there was minimal fibrosis in the region of the gland which was slightly more adherent to the trachea than normal. In the other patient the thyroid presented no abnormal appearances. The histological features in both/

TABLE: XII

Summary of the Results of the Clinical Trial of External Radiation (^{60}Co) in the Treatment of Thyrotoxicosis

RESULTS OF X-RAY THERAPY(^{60}Co) IN 28 PATIENTS (13 PATIENTS TREATED TWICE)

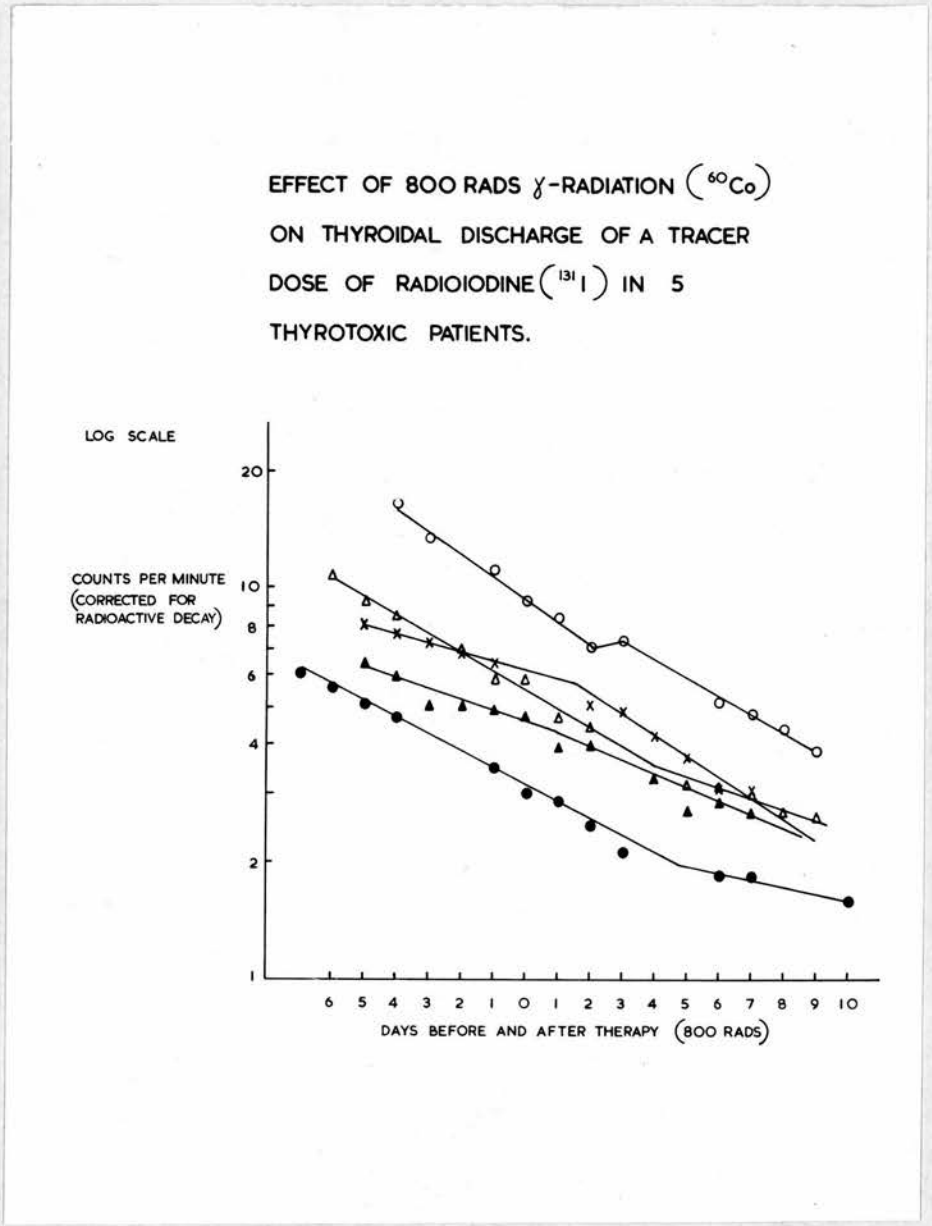
ANTITHYROID DRUGS GIVEN UP TO ONE YEAR AFTER THERAPY. FOLLOW UP 2-3 YEARS

	DOSE OF X-RAYS(^{60}Co) IN RADS.								RESULT	
	115	150	180	400	600	700	800	900	RECURRING TOXICITY	REMAINING EUTHYROID
NUMBER OF PATIENTS								7	7	0
							10		9	1
						1			1	0
					9				8	1
				7					6	1
			1						1	0
		4							4	0
	2								2	0

Fig. 19

Effect of 800 rads -radiation (^{60}Co) on the Thyroidal Discharge of a tracer dose of radioiodine (^{131}I) in 5 thyrotoxic patients. Radioactive counts per minute ($x10^4$) are plotted against days before and after therapy.

Fig. 19

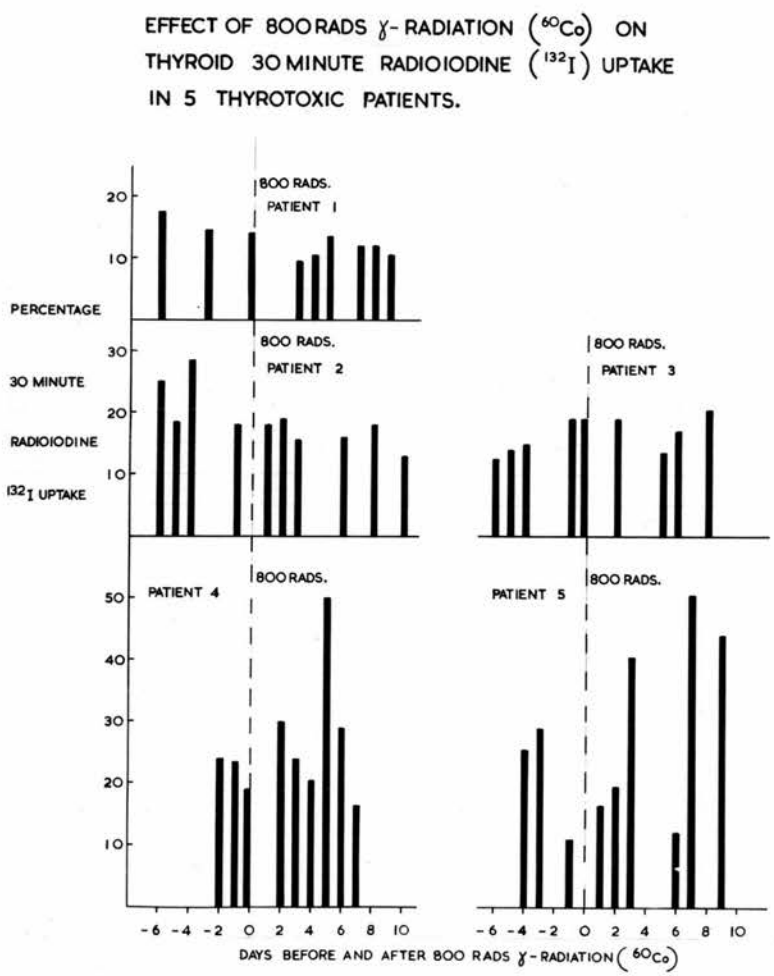


Effect of 800 rads γ-radiation (^{60}Co) on the Thyroidal Discharge of a tracer dose of radioiodine (^{131}I) in 5 thyrotoxic patients. Radioactive counts per minute ($x10^4$) are plotted against days before and after therapy.

both cases were consistent with those of hyperthyroidism which had been modified by antithyroid drug therapy. There were no other unusual features and no evidence of malignancy.

In 5 patients who received 800 rads the effect of radiation on the normal exponential discharge of a tracer dose of ^{131}I was examined along with serial 30 minute ^{132}I uptake studies before and after irradiation. The rate of discharge of ^{131}I was slowed in three patients, accelerated in 1 patient and remained unchanged in the other (Fig. 19 and Appendix Table 22). Thus no consistent effect was observed and the changes themselves were small. Similarly, the pattern of four-hour ^{132}I uptakes after radiation showed no significant deviation from that observed prior to radiation (Fig. 20 and Appendix Table 23). These results are in striking contrast to the effect produced by conventional therapy doses of ^{131}I which accelerate discharge of radioiodine from the gland and greatly diminish four-hour, ^{132}I uptake (Tulloch and Crooks, personal communication).

Fig. 20



The effect of 800 rads γ-radiation (^{60}Co) on the percentage 30-minute uptake of radioiodine (^{132}I) by the thyroid in 5 thyrotoxic patients. The percentage uptake is plotted against days before and after irradiation.

DISCUSSION

Low Dose ^{131}I

Hypothyroidism was not prevented in this study by the use of a small dose ^{131}I and occurred within three years of therapy in 5 patients (17%). In addition, persistence of thyrotoxicosis, recurrence of thyrotoxicosis after a period of euthyroidism and euthyroidism after a period of hypothyroidism, were also found. For these reasons the trial was stopped after only 30 patients had been treated. The variation in the results cannot be explained on the basis of existing radiation dose response studies which have shown similar radiosensitivity of all mammalian cells providing they are well oxygenated (Hewitt, 1962). This suggests that biological factors not related to the millicurie dose administered are important in modifying the results of ^{131}I therapy, e.g. the patchy distribution of ^{131}I in the thyroid (Sinclair, Abbatt, Farran, Harriss and Lamerton, 1955) or vascular damage (Trotter and Willoughby, 1967).

It must be emphasised that formal comparison of these results with those obtained by Smith and Wilson (1967) is best avoided because of certain variables which cannot be accurately assessed. These include the estimate of thyroid gland weight which even in the hands of an experienced observer is a highly subjective exercise. As this estimate is quantitatively important in deciding the millicurie dose/

curie dose of ^{131}I to be given, comparison of the "calculated rad doses" in both studies is unjustifiable. Furthermore in view of the initial patchy distribution of ^{131}I in the thyroid (Sinclair et al. 1955) and subsequent dynamic alterations in this distribution (Levene et al. 1955) the "calculated rad dose" cannot be accepted at its face value even within the one series; it is probably best regarded as a convenient prescription device with no intrinsic therapeutic advantage over arbitrary methods of millicurie dose prescription (Macgregor, 1957). In view of this, the importance of the results reported here lies in the scatter of the results of therapy within the three years of the study. However, the results obtained by McCullagh (personal communication) are not encouraging with regard to the long term incidence of hypothyroidism in patients treated with low doses of ^{131}I. In his series, of 21 patients receiving a dose of 3 m.Ci or less 17 (81%) were hypothyroid ten years later; of ten patients followed for fifteen years after similar doses 9 (90%) had become hypothyroid.

External Radiation Therapy

The results of this study contrast with those obtained in the low dose ^{131}I group. Thyrotoxicosis recurred in all but three of the 28 patients, and the "cure rate" is what would have been expected from treatment with antithyroid drugs alone, Dunlop and Rolland (1950), Solomon, Beck Vanderlaan and Astwood (1953), Trotter (1962), Macgregor/

Macgregor (1963), Reveno and Rosenbaum (1964), Alexander and Harden, (1967). In view of this unequivocal therapeutic failure the trial was discontinued after only 28 patients had been treated.

The failure of a dose of 800 rads to affect thyroid gland function was also shown by the absence of a consistent effect on the exponential discharge of the tracer dose of ^{131}I or on serial half-hour ^{132}I uptakes and is in keeping with the failure of similar doses to affect gland function in rats as shown in Section III.

The absence of histological evidence of radiation damage in the tissue obtained at thyroidectomy is consistent with the findings reported in Section II. It is also of particular interest that Walters, Anson and Ivey (1931) found no histological changes in the thyroids of dogs treated with doses of x-rays similar to those used in the "successful" treatment of thyrotoxicosis at that time. This along with the failure of the therapeutic trial of γ-radiation reported here suggests that the "cures" reported by Hayes (1927), Groover et al (1929) and Soley and Stone (1942) have some other explanation. Spontaneous remission does occur in some 30% of thyrotoxics especially if exophthalmos and goitre are minimal (Ord and Mackenzie, 1897, cit. Wilson, 1967) and selection of cases may have been important in achieving the results reported by the above authors.

Because of the danger of skin and laryngeal damage, doses greater/

greater than 1,000 rads were not employed in this study. Unfortunately, doses of this order had no significant short term effect on the functional cell mass of the thyroid and significant "mitotic death" of the type described by Weinbren et al. (1960) did not occur despite the use of antithyroid drugs which might have been expected to increase the rate of cell division.

An explanation of the results of ^{131}I and external radiation therapy and the differences between them can be found in the results of radiobiological studies in animals. In the experiments reported in Section II there was no evidence of short term cell death in the rat thyroid with doses of x-rays up to 2,500 rads although extreme loss of reproductive integrity (greater than 99.9%) occurred with a dose of 1,000 rads. It was concluded that homogeneous irradiation of the thyroid in a dose sufficient to diminish gland function as a result of cell death would for clinical purposes leave no reproductively intact cells and that a "radiation partial thyroidectomy" with homogeneous irradiation is therefore intrinsically impossible. In addition Al-Hindawi and Wilson (1965) demonstrated shortening of cell life in the thyroid after irradiation with ^{131}I. These observations provide an attractive explanation of the delayed onset of hypothyroidism in thyrotoxic patients successfully treated with ^{131}I therapy as doses of radiation/

radiation sufficient to bring about a rapid decrease in thyroid function are likely to result in thyroid gland failure in the long term due to accelerated cell death and failure of replacement.

In view of the number of successful results of treatment in this and all other reported series there is no doubt that a "radiation partial thyroidectomy" can be achieved with ^{131}I therapy but unfortunately the result is unpredictable in the individual patient. This situation could be explained by the unpredictable distribution pattern of ^{131}I in the thyroid (Levene, Andrews and Kniseley, 1955; Sinclair et al., 1955). A homogeneous distribution of ^{131}I would affect all or most of the thyroid cells resulting in early hypothyroidism. A non-homogeneous distribution of ^{131}I on the other hand would spare areas of the gland in an unpredictable way so that persisting hyperthyroidism, recurrence of hyperthyroidism, the euthyroid state or late hypothyroidism could result. Alternatively varying degrees of vascular damage (Trotter and Willoughby, 1967) may be partly responsible for the unpredictable outcome.

It can therefore be concluded, whatever the total radiobiological explanation, that a predictable radiation partial thyroidectomy is intrinsically impossible. Furthermore it may be anticipated that further reduction of the mean therapy dose would increase/

increase still further the short term incidence of both persisting hyperthyroidism and recurrence necessitating prolonged supplementary treatment with antithyroid drugs (Smith and Wilson, 1967).

CONCLUSIONS

The use of ^{131}I in the treatment of thyrotoxicosis is governed by a consideration of the following factors.

1. The intrinsic impossibility of a predictable radiation partial thyroidectomy in any one patient.
2. The convenience and complete lack of short term morbidity with radioiodine therapy in contrast to surgery.
3. The acceptance of a significant long term hypothyroid rate irrespective of the therapy dose employed.
4. An unwillingness to ablate every patient in view of the lack of information about patient reliability in long term thyroxine replacement therapy and the desirability of inducing "permanent" euthyroidism in terms of a particular patient's life expectancy, without recourse to drug administration in the form of antithyroid drugs or thyroxine replacement therapy.

In view of these considerations the following indications for radioiodine therapy can be drawn up. It must be emphasised, however, that the individual case must be considered on its merits and any particular factor taken into account.

Indications for ^{131}I Therapy in Thyrotoxicosis

In the light of the results reported here there are no indications for the treatment of thyrotoxicosis with external radiation/

radiation alone. In general it is justifiable to use ^{131}I in the treatment of all thyrotoxic patients over the age of 40 years. Post-menopausal women and patients who refuse surgery should also be treated with radioiodine. Other indications include coincident disease such as ischaemic heart disease, cardiac failure, hypertension, chronic respiratory disease, brittle diabetes, psychiatric disease and previous thyroidectomy.

Dose of ^{131}I in Various Categories of Thyrotoxic Patients

In deciding upon the dose of radioiodine to be employed, a large number of factors must be taken into consideration in the individual case. Undoubtedly, the low dose regime described by Smith and Wilson (1967) increases the chance of any one patient remaining euthyroid for the rest of his or her life without recourse to thyroxine replacement therapy. This form of therapy is therefore of great potential advantage in the younger patient, particularly those under the age of 40 years. However, when low doses of ^{131}I are employed many patients require prolonged supervision at a hospital clinic during several courses of antithyroid drugs, whilst awaiting the onset of permanent euthyroidism brought about by the initial ^{131}I therapy. Social factors such as interference with work, care of a young family and distance from the centre are therefore important in deciding upon this form of therapy. Nonetheless, apart from/

from clinical research, this regime has considerable advantages in selected cases. With older patients larger doses of radioiodine can be employed so as to hasten the attainment of permanent euthyroidism while at the same time leaving a reasonable probability that the individual will not become hypothyroid within his life expectancy.

Clearly, further clinical trials are required to define with certainty the points of optimal application of the various dose levels of ^{131}I. In the meantime, however, the following are suggested as an approximate guide for routine patient care. Patients under the age of 40 years should, if circumstances permit, be treated by the low dose regime of Smith and Wilson (1967). Patients over the age of 40 years should receive doses in the range 6 to 12 mCi depending on gland size (Macgregor, 1957), with an arbitrary tendency to the higher end of the dose range with increasing age of the patient. Patients with large nodular goitres are often resistant to radioiodine therapy and may require several doses of the order of 20 mCi. On the other hand, patients who have had a previous thyroidectomy are best treated with small amounts, say 2 to 5 mCi, in order to prevent the high incidence of hypothyroidism which would follow the use of conventional doses. Thyrocardiac patients, i.e. those with heart failure or angina of effort, should receive an "ablative" dose of ^{131}I, i.e. 25 to 50 mCi, in/

in an attempt at rapid and more certain control of their thyrotoxicosis.

In the majority of patients from all categories control of the disease may be more rapidly and predictably achieved with supplementary antithyroid drugs (Greig, Mohamed, Aboul-Khair and Crooks, 1965) starting one week after treatment with ^{131}I and continuing until they are euthyroid. The place of β-adrenergic blocking drugs (e.g. propanolol) as an adjunct to radioiodine therapy requires further evaluation especially in thyrocardiac patients. In those patients who receive an "ablative dose followed by antithyroid drugs, thyroxine replacement should be started as soon as they are euthyroid. This has the twofold purpose of preventing hypothyroidism and allowing a suppression test with a tracer dose of ^{132}I to be carried out. If there is a significant 20-minute uptake by the thyroid (greater than 8%) it indicates that ablation is incomplete and a further large dose of ^{131}I should be given.

Finally, the place of antithyroid drugs alone in the treatment of thyrotoxicosis is being reassessed by Alexander et al (1967). If a permanent remission by this means can be predicted by the tri-iodothyronine suppression test then the indications for both partial thyroidectomy and radioiodine therapy will need to be revised.

As mentioned earlier the decision of how to treat any individual patient/

patient depends on individual circumstances. In particular the above treatment policy assumes the services of an experienced thyroid surgeon. If such is not available, more patients will be treated with radioiodine therapy. In this context it should be noted that in addition to the early complications, surgery results in a significant hypothyroid rate which varies from 5% to 30% in reported series (Hershman, 1966; Wilson, 1967). In Aberdeen, a hypothyroid rate of 38% was found in a random sample of patients followed up 5 to 20 years after a standard radical partial thyroidectomy (Hedley, Michie and Crooks - personal communication). These observations cast the hypothyroid rate after radioiodine therapy in a different light and indicate that surgery is not an ideal alternative to treatment with ^{131}I. Clearly, hypothyroidism following ^{131}I therapy cannot be entirely accounted for by radiation damage.

The above discussion of the indications for radioiodine therapy and the dose of ^{131}I to be employed has been presented on the basis of the degree of probability of any one patient becoming euthyroid or hypothyroid within a certain period of time. It must be emphasised however, that in the individual patient there is no way of predicting the outcome. In particular any patient may become hypothyroid at any time after initial treatment with ^{131}I. The acceptance of a significant hypothyroid rate implies the acceptance of responsibility for detecting and treating these patients and ensuring/

ensuring that they remain euthyroid for the rest of their lives. This is especially the case as the disease is both iatrogenic and amenable to therapy. If no follow-up is possible, thyroid ablation followed by thyroxine replacement therapy might be justified. The latter policy, however shifts the responsibility for the maintenance of the euthyroid state from the doctor to the patient. Unfortunately many patients stop or modify the dose of thyroxine replacement therapy of their own accord (see Section V) and follow-up is necessary to ensure efficient life-long replacement therapy. Accurate assessment of the probability that any particular patient will continue to take replacement therapy is impossible as even intelligent patients are often found at fault. In view of this, the evolution of future management policies for patients with hyperthyroidism, who are candidates for destructive therapy by surgery or ^{131}I, will depend on establishing efficient follow-up schemes. Pilot studies have been carried out in Aberdeen and Manchester on one such method of follow-up. This appears to be practicable, efficient in the detection of hypothyroidism and economical of medical manpower. The results of this study are reported in detail in the following section (Section V).

SUMMARY

Because of the disadvantages of conventional treatment of hyperthyroidism with ^{131}I, two alternative methods of radiation therapy were assessed. One group of hyperthyroid patients received a dose of ^{131}I calculated to deliver 2,400 rads to the thyroid, and a second group was treated by external radiation from a ^{60}Co source, with doses of 115 to 900 rads. Of the 30 patients treated with ^{131}I only 11 were euthyroid 2 to 3 years later. Persisting hyperthyroidism, relapse and hypothyroidism occurred in the remainder. In the patients treated by external radiation, 25 out of the 28 patients relapsed soon after antithyroid drugs were withdrawn and there are therefore no indications for this form of therapy.

It appears that it is impossible to achieve a predictable "radiation partial thyroidectomy" with ^{131}I therapy and that amounts of radiation sufficient to reduce thyroid function to normal will result in a significant risk of eventual thyroid failure. The best compromise appears to be that which attempts to postpone the development of thyroid failure to a time beyond the patient's life expectancy. On this basis a guide to the use of ^{131}I in hyperthyroidism in various groups of patients has been proposed.

SECTION V

A Method of Long Term Follow-Up of Patients Treated with Radioiodine (^{131}I)

INTRODUCTION

Although radiciodine (^{131}I) therapy has many advantages in the management of patients with thyrotoxicosis all workers are agreed that at all therapeutically effective dose levels a significant incidence of late hypothyroidism is inevitable (see Section IV). As many patients treated with ^{131}I are middle-aged at the time of treatment incipient hypothyroidism is often mistaken for the ageing process. Furthermore the apathy of hypothyroidism decreases the chances of spontaneous reporting of the symptoms of the disease to the family doctor. Consequently, many patients reach the stage of advanced hypothyroidism before detection, by which time irreversible damage such as coronary artery disease may have occurred (Bastenie, Vanhaelst and Neve, 1967). Unfortunately neither the hospital nor general practitioner services have accepted formal responsibility for these patients.

Any system designed to deal with this problem must depend upon liaison between the family doctor and hospital services in order that life-long follow-up of every patient can be achieved. It should be able to accurately locate both patient and family doctor and allow assessment of the patient's thyroid state with maximum economy of medical man-power and minimum inconvenience to the patient. A follow-up scheme based on these principles has been put into operation in the North-East of Scotland and its efficacy evaluated. To examine its applicability to a metropolitan area a one/

one year pilot survey of patients treated at the Christie Hospital, Manchester in 1957-58 was carried out. This group was also chosen in order to investigate the difficulties of applying this method of follow-up to patients not under current supervision at the clinic where treatment was initiated.

METHODS

Aberdeen Automated Follow-Up

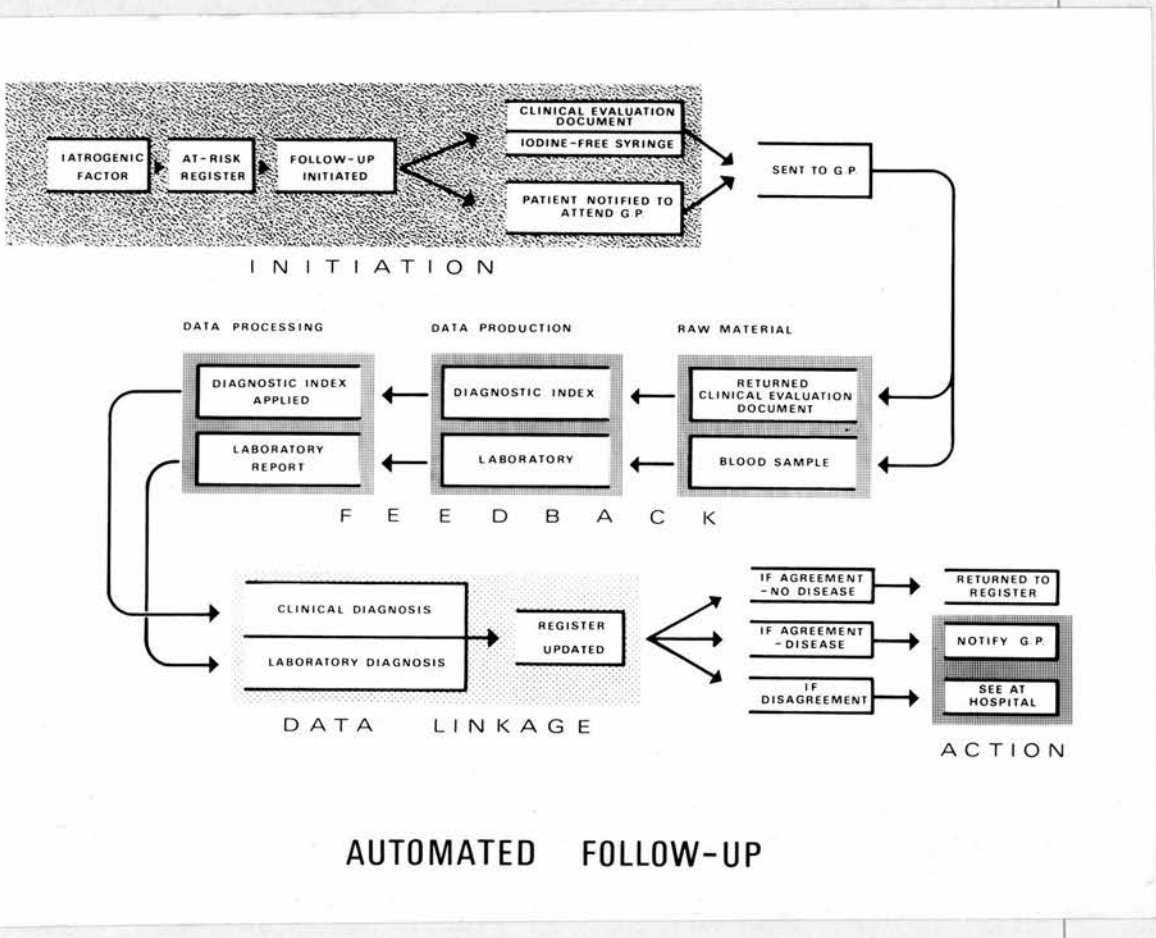
An "at risk" register for patients treated with conventional doses of radioiodine in the Aberdeen area was established in the following way. At the time of treatment a card was completed for each patient with details of the patient's name, address, family doctor and the dates and doses of radioiodine therapy. Similar cards were completed for all patients treated prior to the institution of the "at risk" register. In all, 235 patients had been treated with radioiodine involving 155 general practitioners. Of the 235 patients on the "at risk" register, 109 (46%) were already on thyroxine replacement therapy.

When the patient had been rendered euthyroid, with or without thyroxine replacement therapy, both doctor and patient were asked if they would participate in the life-long follow-up scheme. The family doctors of the 31 patients who, for various reasons (e.g. resident in Orkney), had already been discharged from the clinic were also asked to co-operate in the scheme. If the doctor did not reply to the first invitation to co-operate a "chaser letter", identical to the first, was sent to him. Stated refusal or failure to reply to the first or chaser letters were regarded as non-co-operation. The patients of these doctors continued to be followed at the hospital clinic, since the doctors concerned had no objection to/

Fig. 21

Diagrammatic representation of the mechanics of the Automated Follow-Up for thyrotoxic patients treated with radioiodine (^{131}I).

Fig. 21



Diagrammatic representation of the mechanics of the Automated Follow-Up for thyrotoxic patients treated with radioiodine (^{131}I).

to the conventional hospital follow-up procedure. Each doctor was asked to inform the registry of any change of address of himself or of the patient and an attempt was made via the Executive Council to trace all patients and doctors who could not be contacted by the G.P.O.

The operation of the follow-up scheme is summarised diagrammatically in Fig. 21. If agreement to co-operate was obtained from both patient and family doctor the completed card was removed from the "at risk" register and placed in a "follow-up" register with 12 (monthly) divisions. Once per year each card (patient) was automatically removed from the "follow-up" register by a secretary. The family doctor was sent a special iodine-free syringe cum test-tube (Steriseal Disposable Syringe) for removal of a 10 ml. blood sample with precise instructions for its use. He also received a copy of the Hypothyroid Diagnostic Index shown in Table XIII, (Billewicz, Chapman, Crooks, Day, Gossage, Wayne and Young - to be published). This contains eight symptoms and six signs of hypothyroidism together with the criteria for their recognition. A stamped addressed box for returning the blood sample and completed questionnaire was also provided. At the same time the patient was sent a letter asking him or her to attend the family doctor's surgery within ten days. If no reply was obtained within one month a "chaser letter" identical to the first was sent to both patient/

TABLE: XIII

Hypothyroidism - Diagnostic Index

SYMPTOMS (of recent onset)	Description	SCORE (Ring appropriate value)		
		Present	Absent	Doubtful
1. Diminished sweating	Sweating in a warm room or centrally heated hall	+6	-2	0
2. Dry skin	Dryness of skin noticed spontaneously or on removing clothing, requiring skin cream	+3	-6	0
3. Cold intolerance	Preference for a warm room, extra clothing or bed clothing	+4	-5	0
4. Weight increase	Recorded increase in weight; tightness of clothing	+1	-1	0
5. Constipation	Bowel habits; use of laxatives	+2	-1	0
6. Hoarseness of voice	Speaking voice; singing voice	+5	-6	0
7. Paraesthesiae	Subjective sensations of numbness, tingling, etc.	+5	-4	0
8. Deafness	Progressive impairment of hearing	+2	0	0
SIGNS				
9. Slow movements	Observe patient removing and replacing a buttoned garment	+11	-3	0
10. Coarse skin	Examine hands, forearms, elbows for roughness and thickening of skin	+7	-7	0
11. Cold skin	Compare temperature of examiner's and patient's hands	+3	-2	0
12. Periorbital puffiness	Should oyscure curve of malar eminence	+4	-6	0
13. Slow pulse rate	Count over 30 seconds. Slowing present if under 75/min.	+4	-4	0
14. Slowing of ankle jerk	Elicit with patient kneeling on a chair, grasping its back	+15	-6	0
	Positive and Negative Totals	+	-	
	FINAL SCORE			

Diagnostic Index Score $\leq$ - 25 Euthyroid
-24 to + 19 Doubtful
$\geq$ + 20 Hypothyroid

patient and doctor.

The completed form and blood sample were returned to the secretary for processing. To each of the symptoms and signs on the Diagnostic Index she allocated the scores (discriminant functions) shown in Table XIII. By adding these she obtained a diagnostic score with ranges in which hypothyroidism should be suspected, (Billewicz et al. - to be published). In order to minimise bias by the family doctor in eliciting the various symptoms and signs the scores were omitted from the form sent to him and entered only after the return of the form to the registry. A serum P.B.I. was carried out on the blood sample and interpreted in the light of an established range of normal values (4-8 µg/100ml.). On the basis of both pieces of information the secretary carried out an "act of selective diagnosis" by which the "at risk" patient was allocated to one of three groups, namely euthyroid, hypothyroid or doubtful. If the clinical and laboratory evidence were in conflict the patient was allocated to the doubtful category. At this point a hospital doctor (JRP) sent an individually dictated and signed letter to the family doctor informing him of the results. Patients judged to be euthyroid were returned to the "follow-up" register for repeat screening the following year. Patients classified as hypothyroid or doubtful were asked to attend the hospital clinic for further investigation. This was carried out on clinical grounds with the aid/

aid of the Hypothyroid Diagnostic Index, serum P.B.I. and cholesterol estimations and examination of serial ECG's. Assessment was often facilitated by the possession of "base-line" ECG's, serum P.B.I. and blood cholesterol estimations which had been carried out on all patients prior to their discharge from the clinic. Changes in these parameters in the individual patient could thus be detected. Finally, in difficult cases the response of the various clinical and laboratory parameters to a therapeutic trial with thyroxine replacement therapy was assessed.

Manchester One Year Pilot Survey

A total of 198 thyrotoxic patients treated with ^{131}I at the Christie Hospital clinic between 1957/1958 were studied. These patients were in the care of 190 family doctors at the time of initial treatment. In the first instance the doctors were asked to co-operate in a follow-up scheme in the same way as in the Aberdeen area. If no reply was received a "chaser letter" identical to the first was sent. Only if the doctor indicated willingness to co-operate was a letter sent to the patient asking him to attend the doctor's surgery. Doctors and patients were classified as "untraceable" if they could not be found by the G.P.O. and the letters returned to the sender. No further search was carried out to locate either doctors or patients at this time.

In/

In order to simulate the procedure established in the Aberdeen automated follow-up, all correspondence for the Manchester survey was dispatched from, and returned to, the Christie Hospital, Manchester where the patient had received ^{131}I therapy. The processing of the clinical and laboratory data was, however, carried out in Aberdeen by the same personnel and in the same laboratory as for the Aberdeen automated follow-up.

RESULTS

Aberdeen Automated Follow-Up

Response of Doctors

The response of the 155 general practitioners who were invited to participate in the automated follow-up scheme is summarised in Table XIV. Of these, 143 (94%) agreed to co-operate. Eleven doctors consented after receiving the "chaser letter". Ten doctors did not reply to the first or chaser letters and two replied in the negative. Five doctors who agreed to co-operate were unable to do so because of failure of the patient to attend the surgery.

Response of Patients

The response of the patients to the follow-up scheme is shown in Table XV. Thirty-one patients are not being followed either by the registry or at the hospital clinic because of death (12), follow-up elsewhere, e.g. emigration (9) and failure to attend the family doctor (5). The remaining five patients have been lost to follow-up as a result of changing both their family doctor and their address and failing to register with another doctor under the National Health Service.

From the total of 235 patients, 175 are at present being reviewed annually through the register. Included in this number are 24 patients who have already been reassessed at the hospital clinic because of clinical or biochemical evidence of hypothyroidism, treated/

TABLE: XIV

ABERDEEN AUTOMATED FOLLOW-UP

Response of Doctors

Refusal to co-operate - explicit "no"	2
No reply to first request to co-operate	21
Total "yes" to first request to co-operate	132
Number of chaser letters sent	21
No reply to chaser letter	10
Total "yes" to chaser letter	11
Total Number of doctors willing to co-operate	143(94%)
Total Number of Doctors	155

TABLE: XV

ABERDEEN AUTOMATED FOLLOW-UP

Response of Patients

Patients being followed through register	175
Number of patients dead	12
Patients followed-up elsewhere	9
Number of Patients "lost"	5
Patients affected by non-co-operation of G.P. (Followed at clinic)	12
Patients not attending G.P. for sample	5
Number of patients suspected of hypothyroidism referred to clinic and still attending there	17
Total Number of Patients	235

treated if necessary and placed back in the register for annual review. Seventeen patients who were suspected of hypothyroidism by the postal follow-up are at present attending the hospital clinic for purposes of diagnosis and treatment. An additional 12 patients are reviewed annually at the thyroid clinic because the family doctor preferred the conventional hospital follow-up procedure.

The Operational Economy of the Automated Follow-Up Scheme

As a result of the automated follow-up scheme it has been necessary to see only 53 of the patients at the clinic over the past $2\frac{1}{2}$ years. If the scheme had not been operating annual review would have necessitated at least 480 (2.5 x 192) patient visits to the clinic. Thus, in terms of patient load per annum (strictly not equivalent to clinic visits per annum) there has been a reduction of 89% in the demands made upon the hospital clinic. It is anticipated that the clinic work load will maintain itself at approximately 10% of its original level. In lieu of this a hospital doctor spends one hour each month supervising the register and the family doctor performs approximately $1.27\left(\frac{175}{138}\right)$ venepunctures per annum in addition to completing the Diagnostic Score sheet. The estimated work load on the family doctor is approximately 10 minutes per annum.

Efficiency of the Follow-Up Scheme in the Detection of Hypothyroidism

It can be seen from Table XVI that over the first two and a half years since the automated follow-up was begun, 41 patients have been brought back to the hospital clinic suspected of hypothyroidism.

Eighteen of these 41 patients were supposedly on adequate thyroxine replacement therapy for established hypothyroidism. In this group, 9 were suspected on the grounds of the Hypothyroid Index score, 4 because of a low serum P.B.I. and 5 from both the Diagnostic Index score and low serum P.B.I. Stated in another way, 14 patients had suspicious Diagnostic Index scores and 9 patients a low serum P.B.I. Of these 18 patients, 14 were finally diagnosed as hypothyroid at the hospital clinic. Had the automated follow-up been carried out using the serum P.B.I. alone as the indicator of hypothyroidism, 7 of the patients finally diagnosed as hypothyroid would have been missed. If the Diagnostic Index had been used alone two patients would have been missed. Thus, although the numbers are small, in the group of patients on thyroxine replacement therapy the Hypothyroid Index score appears to be the more efficient indicator of hypothyroidism.

There were 23 patients suspected of hypothyroidism who had not previously been on thyroxine replacement therapy. Five were suspected by the hypothyroid Diagnostic Index score, 8 from a low serum P.B.I. and 10 by both the Diagnostic Index score and serum P.B.I./

TABLE: XVI

ABERDEEN AUTOMATED FOLLOW-UP

Efficiency of Hypothyroid Index Score and Serum PBI in the Detection of Hypothyroidism by Automated Follow-Up

Previous Thyroxine Replacement Therapy	Suspected of Hypothyroidism by -			Total Number of Patients Suspected	Number Finally Diagnosed Hypothyroid at Clinic*
	Score Alone	PBI Alone	Both Score and PBI		
Yes	9	4	5	18	14 (72%)
No	5	8	10	23	11 (48%)

*On basis of hypothyroid diagnostic index score, PBI, serum cholesterol, ECG and response to trial of thyroxine replacement therapy.

P.B.I. Thus, in this group, 15 patients had an abnormal Diagnostic Index score and 18 a low serum P.B.I. Of these 23 patients, 11 were subsequently shown to be hypothyroid. Only one patient would have been missed if the automated follow-up had been carried out using the serum P.B.I. alone. Three patients would have been missed if the diagnostic score had been used alone. Thus, in those patients not on thyroxine the serum P.B.I. appears to be the more efficient indicator of hypothyroidism.

A measure of the consistency of the automated follow-up is to be found in the fact that since the follow-up scheme was initiated 35 of the patients on no previous thyroxine replacement therapy have been screened twice. Of these only four were suspected of hypothyroidism on the second screening who had been considered euthyroid on the first. Two of these were subsequently found to be hypothyroid after assessment at the hospital clinic. This number is consistent with the annual increase in the cumulative hypothyroid rate with time after initial therapy reported in other series: (Smith and Wilson, 1967).

Manchester One Year Pilot Survey

Response of Doctors

The results are summarised in Table XVII. Of the 190 doctors who were invited to participate in the automated follow-up scheme 73 agreed to do so after the first request. Following a chaser letter/

TABLE: XVII

MANCHESTER DOCTOR SURVEY

	Number of Doctors
Willing to co-operate	78
Willing but Unable to Co-operate) Patient dead	16
Willing but Unable to Co-operate) Patient untraceable	33
Willing but Unable to Co-operate) Patient being followed elsewhere	6
Willing but Unable to Co-operate) Patient not attending surgery	11
Untraceable, retired or dead	17
Not willing to co-operate	29
Total	190

letter this number increased to 89. Eleven doctors who agreed to co-operate could not do so because their patients did not attend the surgery. The final effective co-operation therefore was 78 out of 190 doctors, i.e. 41%. However, outright non-co-operation was found in only 15% (29) of general practitioners. The remaining 44% of family doctors were not in a position to co-operate fully although their replies indicated a willingness to do so had this been possible. If the 17 doctors who were untraceable, retired or dead are excluded from the analysis a total of 134 doctors out of 173 (83%) either co-operated or showed willingness to do so. If the 83 doctors who were not in a position to co-operate are excluded, an effective co-operation rate of 73% (78 out of 107) is obtained.

Response of Patients

The results relevant to the efficiency of follow-up of the patients is shown in Table XVIII. Of the 198 patients in this group only 80 were finally assessed via the automated follow-up scheme. Seventeen patients had died, 7 were being followed elsewhere and 30 patients were not screened because of non-co-operation on the part of the family doctor. Thirty-five patients whose previous family doctors were agreeable to co-operate could not be traced by the G.P.O. Seventeen patients were excluded by virtue of the fact that their family doctor at the time of therapy (1957-58) could not be traced, was dead or retired. Finally 12 patients failed/

TABLE: XVIII

MANCHESTER PATIENT SURVEY

		Number of Patients
No attempt at location	G.P. non-co-operation	30
	G.P. untraceable, retired or dead	17
Patients not located		35
Patients dead		17
Patients located	Followed through register	80
	Followed currently elsewhere	7
	Not attending G.P. for blood sample	12
Total		198

failed to attend their family doctor for the appropriate clinical examination and blood sample.

By the same criteria as in the Aberdeen Follow-up, 25 patients (out of 80) were suspected of hypothyroidism and have been referred back to a hospital consultant for further assessment. The high percentage of suspects (31%) in this series is probably due to the long period (8 years in most cases) since cessation of hospital supervision. During such a period significant numbers of patients inevitably became hypothyroid, irrespective of the initial therapy dose. The low detection rate in the absence of efficient follow-up is reflected by the fact that only 16 of the 80 patients screened (20%) were known to be on thyroxine replacement therapy. This contrasts with the much higher hypothyroid rate in all other series where conventional millicurie doses of ^{131}I have been employed, e.g. 40% eight years after therapy in the series reported by Crooks (1965).

The assessment of the 25 patients suspected of hypothyroidism was carried out by a number of different consultant physicians at various clinics in the West Midlands. Because of the lack of standardisation the results of these assessments are not presented here. The main objective of the survey viz. the investigation of the operational difficulties inherent in the retrospective application of the Aberdeen Automated Follow-Up Scheme to a metropolitan area had, however, been achieved.

DISCUSSION

The Automated Follow-Up Scheme has been highly successful in the Aberdeen area which extends to the North of Scotland and Orkney and Shetland. In these circumstances this type of follow-up is extremely convenient and time-saving to the patient and doctors alike. With 90% of general practitioners co-operating the patient load on the hospital out-patient clinic was reduced by 89%. The fact that the entire operation is run by a secretary even to the point of making a "selective diagnosis" on the basis of numerical information from the Diagnostic Index score and serum P.B.I. represents a further economy in medical man-power.

With regard to the mechanics of the follow-up scheme it is clear that clinical assessment based on the Hypothyroid Diagnostic Index (Billewicz et al., 1968) along with one biochemical index such as the serum P.B.I. is an efficient and consistent method of detecting undiagnosed hypothyroidism even in the hands of staff without medical training. As might have been anticipated, the diagnostic index score is most efficient in those patients on thyroxine replacement therapy because of the distortion of the normal range of serum P.B.I. even by inadequate or intermittent thyroxine administration. On the other hand, it should be noted that the Hypothyroid Diagnostic Index employed here was designed to be most efficient in the diagnosis of spontaneous hypothyroidism which/

which probably pursues a different clinical course from iatrogenic hypothyroidism. This may account for the finding that the serum P.B.I. was a more efficient indicator of hypothyroidism than the Diagnostic Index in those patients not on thyroxine therapy. Clearly, however, the use of two indicators offers an insurance against failure of one of them in any particular case.

The fact that 18 out of a total of 109 patients on thyroxine therapy were suspected of hypothyroidism of whom 14 (13% of the total) were ultimately shown to be hypothyroid indicates that a significant proportion of patients cannot be trusted to adhere to long term replacement therapy. Twenty-three patients out of a total of 126 who were not on thyroxine replacement therapy were suspected of hypothyroidism of whom 11 (8.6% of the total) were ultimately shown to be hypothyroid. Taking both groups together 11% of all radioiodine treated patients in the Aberdeen area had been shown to be hypothyroid over the past $2\frac{1}{2}$ years. In view of the predominantly prospective nature of this follow-up it is likely that, at the very least, 11% of all radioiodine treated patients are hypothyroid in varying degrees. As approximately 160,000 of thyrotoxic patients have been treated with ^{131}I, (Greig, 1966) there therefore exists a large morbid population who are potentially curable.

The gravity of the situation is emphasised by consideration of the/

the results of the Manchester pilot survey. Of these patients treated ten years previously at the Christie Hospital, Manchester, only 44% (87) could be followed-up by the registry. Here the intrinsic problems of diagnosing incipient hypothyroidism are amplified by the large number of patients treated in this centre (approximately 500 per annum) combined with a rapidly moving population with consequent increased difficulty in tracing patients. These findings are in keeping with the information contained in the 1961 census migration tables for this area which indicate that approximately 18% of this population change their address within 2 years. Nonetheless, 84% of the family doctors in this area indicated their willingness to co-operate in such a follow-up scheme. It is likely that even more would have been willing to co-operate had the scheme applied prospectively.

CONCLUSIONS

To be fully successful life-long follow-up must be initiated prospectively at the time of therapy. Any delay decreases the possibility of efficient supervision. Nevertheless as a public health measure the problem of tracing all ^{131}I treated patients should be tackled as quickly and vigourously as possible in view of the fact that many of them are suffering from a potentially curable iatrogenic disease.

Neither the hospital consultant nor the family doctor is on his own well equipped to carry out life-long follow-up of this type. This function can probably best be carried out on a regional basis with an organisation designed to bring about a greater degree of co-operation between the family doctor, hospital and laboratory services. A register of "at risk" patients maintained in each centre would obviate the difficulties of change of address of the patient, or change or death of the family doctor or hospital consultant. At the same time the family doctor remains responsible for the overall care of his patient and carries out the follow-up with the aid of the hospital laboratory and administrative services. The need for hospital admissions and out-patient clinic attendances is greatly reduced and so prevents the "snowballing phenomenon" which is the inevitable result of cumulative long term follow-up at hospital out-patient clinics. Further economies in medical man-

power/

power derive from the fact that the entire administrative work load can easily be carried by a secretary with minimal supervision from the hospital consultant and as each family doctor has only a few such patients there is no undue increase in his commitment.

This type of patient-care might well be employed to solve problems inherent in other forms of treatment resulting in long-term iatrogenic morbidity, e.g. anaemia and bone disease following partial gastrectomy (Crooks, Clark, Amar and Coull, 1965). The precise details of such follow-up schemes would be designed to meet particular problems in each disease but the success of this project is an encouragement to the development of diagnostic indices for other disease processes. Finally, it emerges that the efficiency of life-long follow-up should be a prime determinant in deciding upon any therapeutic procedure which produces significant long-term morbidity.

The potential increase in administrative work-load may seem formidable if this philosophy is carried to its logical conclusion. However, if Fig. 21 is re-examined it can be seen that the Automated Follow-Up Scheme was designed with future computer involvement in mind. The transfer from manual to computer administration of the scheme is now taking place and is being extended to include surgically treated thyrotoxic patients. The efficiency of both clinical and experimental medicine is under a constant threat from excessive/

excessive routine and administrative commitments. The application of computer techniques to these problems should enable the individual doctor to concentrate more on the tasks for which he was trained with consequent benefit to the patient.

SUMMARY

In recognition of the high cumulative incidence of hypothyroidism following radioiodine treatment with thyrotoxicosis, the unreliability of patients in long term self administration of thyroxine and the difficulties in diagnosing hypothyroidism, an automated life-long follow-up scheme for patients treated with radioiodine has been evolved. This is based on the clinical diagnosis of hypothyroidism with the aid of a computer derived "Hypothyroid Diagnostic Index" (Billewicz et al. - to be published) and a serum P.B.I. estimation. In the Aberdeen area a prospective study has shown this to be an efficient and consistent method of detecting undiagnosed hypothyroidism, with maximal economy in medical man-power. A one year pilot survey in the Manchester area has demonstrated clearly the need for such a scheme and the difficulties involved in its retrospective application. It is proposed that responsibility for such patients should be assumed by the medical services of an area, integrating the family doctor, hospital and laboratory services in order to achieve the highest possible standard of life-long patient care.

SECTION VI

Some Theoretical Considerations in Quantitative Histology.

INTRODUCTION

Certain quantitative histological techniques are concerned with the determination of numbers, volumes and surface areas of tissue structures. In the course of the investigations reported in Sections I and II, it was found that while these methods are in common usage some of the theoretical assumptions on which they rest have never been rigorously established and are, in some cases, unjustifiable. Moreover, the practical circumstances in which these techniques are and are not valid have not been strictly defined, with the result that they may be in danger of misapplication. This study was undertaken in an attempt to improve the theoretical understanding and to define the practical limitations of these methods.

Randomness in Organised Tissues

All the methods to be considered are statistical in nature and therefore depend on some assumptions about randomness. Now tissue components cannot interpenetrate and their distribution cannot therefore be completely random, quite apart from any inherent tissue regularities. Fortunately however, in order to obtain randomness of sampling procedure, it is sufficient to choose the measurement position on the given histological section and the orientation of the section at random. It is not always necessary to do both however.

Volume Estimations

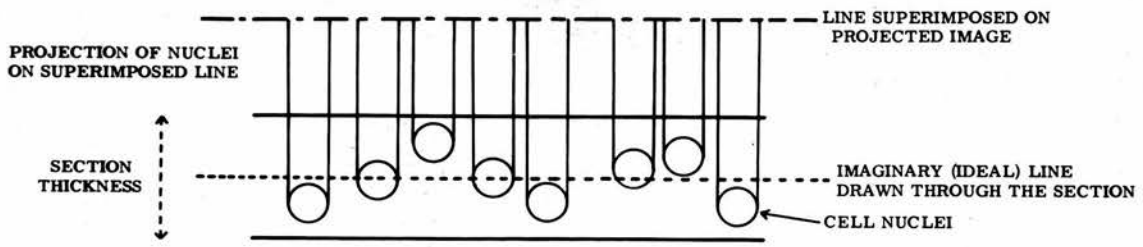
All existing methods for estimating the mean volume (relative and absolute) of distinct tissue structures depend on the projection of an image of a stained microscopic section onto a plane surface, such as a screen or the retina of the eye. It has been stated or implied that the relative total areas on that plane surface occupied by the images of the various components are directly proportional to the relative total volumes of those components: Chalkley (1943), Tala (1952), Uotila and Kannas (1952), Santler (1957), Hally (1964). Consequently methods of estimating these relative areas have been assumed to yield estimates of the relative volumes of tissue components.

Tala (1952) superimposed two random lines on to the projected image of a stained tissue section, and derived the relative component volumes by assuming them to be proportional to the relative values of the sums of the segments of the lines lying over the images of these components. Chalkley (1943) and Hally (1964) superimposed a set of random points over the projected image, and assumed the relative component volumes to be proportional to the relative number of points which lie over the various components. All these assumptions which equate relative areas with relative volumes are true only under certain conditions which strictly speaking cannot be achieved. Unless the theoretical considerations underlying these methods/

methods are understood they may be used in inappropriate situations and invalid results obtained.

Geologists have long used methods similar to those mentioned above for estimating the relative volumes of different mineral species in rock samples: Chayes (1954). The plane surface exposed by cutting through a rock is examined by reflected light and the opacity of the specimen to light ensures that the section is, effectively, very thin. A histological section, however, is three-dimensional and contains within it three-dimensional tissue components. When a stained microscopic section is examined it is the two-dimensional projected image of these three-dimensional components on a plane surface which is actually visualised. Chalkley (1943), Tala (1952) and Hally (1964) by comparing their results with those obtained by planimetry have demonstrated that their methods do measure the relative areas of the projected plane image. These areas do not, however, always correspond to the relative volumes of the three-dimensional tissue components. One of the reasons for this is shown in Fig. 22 in which the number and length of segments of two lines intercepting cell nuclei are compared. One imaginary line passes through the tissue (the ideal line) and the other (the practical line) is superimposed on the projected image. The diagram is, of course, a two-dimensional representation of a three-dimensional situation. If it is assumed that/

Fig. 22



Comparison of the Estimates of Relative Nuclear Volume obtained by an ideal line drawn through a tissue section with that obtained by a line superimposed on the projected image of the section.

that for the ideal line passing through the tissue the relative total volume of nuclei is equal to the fraction of the length of the line lying within nuclei (see Proof A) then clearly the superimposed line gives a much higher (and erroneous) value for the relative total volume of cell nuclei. This argument applies to any tissue component and a correction factor is required for this "inclusion effect". It can be seen that the thinner the section the more closely it approaches the ideal plane of zero thickness whose projection corresponds to the plane itself, and for which the ideal and superimposed lines will therefore also correspond.

Two general methods for meeting this difficulty of estimating the relative volumes of three-dimensional structures from their two-dimensional plane projected images are discussed below under the headings "thin sections" and "thick sections".

"Thin" Sections

The papers of Chalkley (1943), Tala (1952) and Uotila and Kannas (1952) correctly state that if an imaginary random line is drawn through a tissue, the relative values of the sum of its segments lying within distinct components is proportional to the relative volumes of those components (see Fig. 22). Chalkley goes on to say that the relative volumes of tissue components is also proportional to the relative numbers of imaginary random points distributed/

distributed in space which coincide with the various components. Similarly if an ideal plane of zero thickness is drawn through the tissue, the relative total area of the plane lying within the various components will be proportional to the relative total volumes of those components: Proofs of these three propositions are provided later (Proofs A, B and C).

If it were possible to cut and stain infinitely thin sections, both the section and its projected image would correspond to the ideal plane. In this event, planimetry, the random line method of Tala (1952) and the random points method of Chalkley (1943) and Hally (1964) would be absolutely valid for estimating relative volumes. Infinitely thin sections cannot be obtained, but if the section were "very thin" relative to the structure whose relative volume was being determined, it is reasonable to assume that a fairly accurate estimate would be obtained. One can approximate to this situation in two ways (A and B).

(A) Sections are cut as thinly as possible relative to the dimensions of the structures whose relative volumes are being estimated. A random line or random points can then be superimposed on the projected image, and values for the relative component volumes derived. The absolute total component volumes for the whole organ can then be calculated providing the volume of the latter is determinate,

i.e./

$$\text{i.e. } V_a = V_t R$$

where V_a is the absolute total volume of a tissue component, R its relative volume and V_t the total organ volume. Furthermore, the mean volume of any structure, e.g. cells or nuclei, can now be calculated if their total number is determined

$$\text{i.e. } V_m = \frac{V_a}{N_t}$$

where N_t is the total number of any particular tissue component in an organ and V_m its mean volume.

This method is probably of greatest value in examining non-homogeneous tissues such as the thyroid where relatively large volumes in any one part of the gland may be occupied by one component, e.g. large acini filled with colloid may predominate in one part of the gland, and large volumes of cytoplasm formed by dense aggregates of cells in another. Such components have a large mean diameter relative to the section thickness so that ordinary (e.g. 6μ) sections can be employed. Moreover with low power magnification, and hence large fields, whole sections can be scanned quickly with the assurance that truly representative samples are being taken.

(B) This method makes use of the small depth of focus possessed by lenses of high numerical aperture (N.A.). For example, an oil immersion objective with N.A. = 1.25 used with a x10 eyepiece has a depth of focus of only 0.5μ ; (Glasser, 1944). By this means one effectively visualises a very thin slice of tissue. In these circumstances/

circumstances the projected image approximates very well to the ideal plane and the methods of Tala (1952), Chalkley (1943) and Hally (1964) are valid for estimating the relative volumes of tissue components whose mean diameter is large relative to the thickness of the slice of tissue visualised. This technique makes easily available a very thin stained slice of tissue for volume estimations. Unfortunately it can only be employed for examining fairly homogeneous tissues in which sufficient numbers of the various components in representative proportions can be seen in one high power field. For non-homogeneous tissues the number of fields required to scan even one entire section would be formidable. Examination of fewer fields would yield results in which one could have little confidence. As Chalkley (1943) points out, this method is suitable for estimating the relative volumes of nuclei and cytoplasm. As nuclei are often spherical or ellipsoidal their absolute mean volume can be calculated, and hence the absolute mean volume can be calculated, and hence the absolute mean cytoplasmic and cell volumes.

"Thick" Sections

This approach involves the derivation of a correction factor which would enable relative volume estimations to be made from "thick" microtome sections, i.e. where the section thickness is large compared to the size of the component whose relative volume is/

is being estimated.

When correcting for section thickness there are two major effects to be taken into account. The first of these is an "inclusion effect" which arises from the fact that when a particular tissue component is cut by either the upper or lower face of a histological section, the area which is seen on, say, a microscope projection screen, is not the area cut by the section face (i.e. the ideal plane) but the projected area of that part of the component within the section. This effect is even more pronounced if the component is contained entirely within the section. Secondly when the component size, component concentration or section thickness are large enough, screening effects are commonly observed when the projected image of one component partly or wholly overlies the projection of another. The "inclusion effect" tends to increase the estimate of the relative volume of a component while the "screening effect" tends to minimise it.

The following equation has been derived to take these two factors into account (see Proof D).

$$1 - Ro = (1 - R)e^{\frac{-RT}{\bar{L}}} \qquad (1)$$

where Ro is the observed ratio of the projected component area to total area, R is the ratio of component volume to total volume, T is the section thickness and $\bar{L}$ is the mean length of a random chord of/

of the particles. The equation applies to a collection of congruent convex particles. Ro, T and $\bar{L}$ are experimentally determined quantities. From (1) R can be found by an iterative procedure and a first approximation is given by

$$R = \frac{Ro.\bar{L}}{T + \bar{L}} \qquad (2)$$

In the case of spherical particles the mean chord $(\bar{L}) = \frac{2D}{3}$ and (1) and (2) become

$$I - R_o = (I - R)e^{-3RT/2D} \qquad (3)$$

and

$$R = \frac{2R_o.D}{3T+2D} \qquad (4)$$

It should be noted that with "thick" sections the determination of the relative volumes of two distinct tissue components with differing diameters is not straight forward. This is because the particle size and therefore the screening and inclusion correction factors differ for the two components. Thus if T is large relative to the mean diameter of two components their volumes relative to the whole must be determined separately and thence their volumes relative to one another. It must also be emphasised that formula (1) is only an approximation as the analytical difficulties have prevented an exact solution of this problem. It is probable that a "Monte Carlo" computer simulation experiment could assess its reliability in any particular case.

Number Estimations

Abercrombie (1946) deals with the theory and practice of the enumeration of spherical and ellipsoidal components (e.g. nuclei) in tissue sections. This has been extended to deal with components of any shape in Proof E. The problem, briefly stated, is that when the number of any tissue component is counted in a stained tissue section, the estimate is invariably too high. A correction factor has to be introduced to allow for the fact that any section will include fragments of components cut through by the microtome which are not wholly contained within the section, i.e. an "inclusion effect".

In the case of a random distribution of component particles in the form of equal spheres it is not difficult to see that N, the true count, is related to N' the observed count by the equation

$$N = N' \cdot \frac{T}{T+D} \qquad (5)$$

where T is the section thickness and D is the diameter of the spheres. This follows because any sphere whose centre is within a distance $\frac{D}{2}$ from a section face must have part of its volume within the section volume and will therefore be accounted as a particle belonging to the section. In other words we are counting all spheres whose centres lie within an "effective" section thickness $T + 2.\frac{D}{2}$ (Proof E).

It

It can be shown that, for a random distribution of particles of the same size and shape, the appropriate formula is

$$N = N' \cdot \frac{T}{T+\bar{L}} \qquad (6)$$

where $\bar{L}$ is the mean random chord length for the particle (Proof E).

The effect of screening on the particle count is more difficult to assess than in the case of volume estimation where a definite statistically based measurement procedure is followed. Counting procedures depend on a visual examination of selected fields of view and the eye can readily detect when two particles are overlapping and therefore avoid the mistake of counting them as one. It is possible that an electronic method of counting, unless adequately sophisticated, could be defective if no correction for screening were used, but the precise effect of screening would depend very much on the type of electronic scanning system employed. Because of this no attempt has been made to construct a screening connection for number estimations.

Determination of the Ratio of Volume to Surface Area of Tissue Components

Cornfield and Chalkley (1951) state the result

$$rh/c = 4 \text{ volume/surface area}$$

where r is length of a line thrown many times at random over many randomly cut facets of a three-dimensional structure, h is the number of/

of "hits" scored by the ends of the line over a cut area, and c is the number of times the line cuts the surface of the figure. A proof of this theorem is given by Cornfield and Chalkley (1951). A simpler proof is provided here (Proof F).

Although this result is correct when a line is randomly placed across the randomly cut surfaces of a polyhedron it takes no account of projection. In other words the projection of a cut polyhedron does not correspond to the area exposed by cutting. It is reasonable to assume that a microtome knife cuts randomly through the structures contained in a tissue but only if the section is a "very thin" one will the projected areas approximate to the actual areas of those structures through which the knife has passed.

In view of these arguments, this method should be used only if the section is "very thin" relative to the structure whose volume/surface ratio is being investigated. Identical objections apply to the application of Short's result (1950-51) to volume/surface estimation in tissue sections. It should be noted that both Cornfield and Chalkley's (1949) and Short's (1950-51) results are modifications of Buffon's needle problem (Kenny and Keeping, 1951). Another interesting adaptation of this fundamental theorem is that of Loud (1962) in which he measures the length of the cell endoplasmic reticulum in electron micrographs of ultra-thin sections.

If the volume/surface area ratio (Q/4) of a tissue component is/

is determined as above and the mean cell volume (V_m) calculated by the methods already discussed, then the mean cell surface area (S_m) can be calculated from Cauchy's result (vide infra),

$$\text{i.e.} \quad S_m = \frac{4V_m}{Q}$$

Cauchy's Theorem

Cauchy (1908) and Vouk (1948) have shown that the relationship between the surface area (S) of a non re-entrant polyhedron and the mean area of its projected image ($\bar{A}$) is given by the equation

$$S = 4\bar{A}$$

If the shape of the polyhedron is determinate, the surface area (S) can be related to its volume (V), and hence $\bar{A}$ can be related to V through S.

Chalkley (1953) points out from a review of the literature that, in compact tissues at any rate, the cells may approximate to the Archimedean tetrakaidecahedron whose surface is composed of 6 squares and 8 hexagons. The relation of surface area to volume is known for this figure so that both can be calculated from a knowledge of its projected area. However, it is not justifiable to assume that, after the microtome knife has cut randomly through a tissue that the parts of the cut cells contained within the section are tetrakaidecahedrons. Their shapes are in fact indeterminate, and certainly no longer correspond to the original cell shape whatever that/

that may be. Thus Cauchy's theorem is inapplicable to histological sections as cut tissue components cannot have the same shape and size as the components themselves.

Practical Considerations

With regard to the choice of methods, whether in the estimation of relative volumes, numbers or surface areas, it is impossible to select one method which is invariably superior to the other in all situations.

In relative volume estimations there is no doubt that Chalkley's (1943) random point method is superior to Tala's (1952) random line method with regard to ease of performance. This is because it is easier to decide if a point lies over a structure than to make many linear measurements across structures whose boundaries are often indistinct. Chalkley's (1943) original technique is nonetheless inconvenient as it involves sticking hairs on to the microscope eyepiece so that they project into the field of vision. The random points are formed by the ends of the hairs. In the studies reported in Section I the points of intersection of the lines of a regular grid eyepiece graticule were used as a lattice of random points with respect to the tissue components. In this case randomness obtains because the positions and orientation of the grid relative to the cell distribution are considered to be random. Alternatively,/

Alternatively, graticules with engraved random points are commercially available.

One advantage of the "grid method" is that in the process of obtaining the relative component volumes in any one microscope field, the standard deviation for the whole section can also be obtained (see Proof C and Hally, 1964). By comparing this theoretical standard deviation with that obtained from making observations at many places in a section the reliability of the particular technique being used can be checked. The two values will correspond only if the method of estimation is reliable and if the one field initially examined is representative of the whole section, e.g. with a homogeneous tissue such as liver. A regular grid graticule may also be statistically superior to a random points graticule when the distance between the points of intersection of the grid is such that when one point lies over the projection of a component, the immediately adjacent points do not. That is, when the distance between points is greater than the mean component diameter and less than the mean distance between components. Fulfilment of these conditions will yield valid results from fewer observations (Harding - personal communication) and this fact was exploited in the investigations reported in Section I.

In the case of a non-homogeneous tissue a single representative sample can only be obtained by examining a large microscope field which/

which usually means low resolution and magnification. The difficulty of small magnification can be overcome by using a projecting microscope. With this instrument the large field obtained with a low power objective can be magnified many times simply by increasing the distance between the microscope and the projection screen. If a high degree of resolution of detail is required, this technique is of no value, and in order to ensure adequate sampling one can only resort to the examination of many fields with an objective with higher resolving power. In general then, in order to cut down the number of samples (fields) to be taken, one would use an objective which will provide the largest possible field of view compatible with the minimum degree of resolution required.

In view of the above discussion it can be seen that the method chosen for estimating volumes from histological sections will depend on several factors. These include the mean component diameter, the mean section thickness and the degree of accuracy desired. The general character of the tissue under consideration should be carefully examined in order to ensure that the theoretical model approximates to the real situation. For example care should be exercised in defining the mean component diameter. The case of discrete non-contiguous components such as cell nuclei is straight forward and the correction factor derived in Proof D can be applied using the mean nuclear diameter. However where aggregates of cells occur,/

occur, it is the mean diameter of these aggregates which must be taken into account and not the mean diameter of the individual cells themselves. This point is illustrated by the thyroid follicular cells which form a "capsule" enveloping the acinar colloid. Strictly speaking the relative volume of these cells can only be obtained by estimating separately the relative follicular and acinar volumes by substitution of the mean follicular and acinar diameters in the correction equation. The difference is equal to the relative total follicular cell volume. Alternatively as the mean follicular and acinar diameters are often of the order of 50μ to 100μ a 5μ section is relatively "very thin" and Method A is applicable.

Finally it is important to accept the fact that true randomness in the statistical sense is not a feature of organised tissues. Consequently, care and commonsense are essential to obtain meaningful results in a theoretically intractable situation.

PROOFS

Proof A: Line intercept method for estimating relative volumes

If a straight line is drawn through a volume of tissue (V) of any shape whatever, containing a random distribution of, say, nuclei of various sizes, shapes and orientations, then we require to prove that

$$\frac{l}{L} = \frac{v}{V} \qquad (7)$$

where v is the total volume of nuclei, L is the length of line within the tissue and l is the sum of the intercepts of the nuclei on the line.

The proof of this is almost self-evident. If we consider the line to be the axis of a long narrow cylinder of small cross-section then the length ratio in (7) is now a volume ratio, a ratio of a small volume of nuclear material to a small volume of tissue. This ratio, in other words, is a sample of the volume ratio we desire to measure. If we imagine this long, thin rod to be thrown randomly within the tissue volume then the ratio of the sum of the nuclear volumes to the sum of the tissue volumes must approximate to the ratio of nuclear volume to total tissue volume. Another way of approaching this problem is to remember that any point, or tiny volume, of the tissue is as likely as any other to be included within the volume of the rod. Since the nuclei are randomly scattered within the tissue space the result is intuitively obvious. This,

of/

of course, gives no estimate of the expected standard deviation of this method of estimation. In practice a succession of readings for various random positions of the rod will provide an experimental value for the standard deviation.

Proof B: Plane intercept method for estimating relative volumes

We consider again the same distribution of nuclei in tissue and a random plane cutting through the tissue.

If "A" is the area of the plane, and "a" is the total area of intersection with the nuclei, we wish to show that

$$\frac{a}{A} = \frac{v}{V} \qquad (8)$$

The proof is analogous to the proof given above by considering the plane replaced by a thin lamina.

Proof C: Random points in space method for estimating relative volumes

As before, we consider a volume V of tissue containing a random distribution of nuclei which may be of various sizes, shapes and orientations. If a point is taken at random in this volume, the probability that it will be inside a nucleus is $\frac{v}{V}$ where v is the total volume of nuclei. If any other point is taken we have the same probability associated with it. If N points are taken then the probability that r points lie in nuclei is given by the $(r + 1)^{th}$ term of the binomial expansion $(q + p)^N$ where $p = \frac{v}{V}$ and $q = 1 - p$.

The mean of this distribution is N_p and the standard deviation is $\sqrt{Npq}$. In this case the mean number of points lying inside nuclei will be $N.\frac{v}{V}$ with a standard deviation $\sqrt{N\frac{v}{V}(1-\frac{v}{V})}$.

In the two-dimensional case where a rectangular lattice overlies the projected image of a section of tissue, a similar analysis shows that the fraction of points lying over nuclei is equal to $\frac{a}{A}$ where a is the projected area of nuclei in a section area A. The conditions under which this area ratio is equal to a volume ratio are treated in the text and in Proof D.

Proof D: Correction Factor for Relative Volume Estimations from Thick Sections

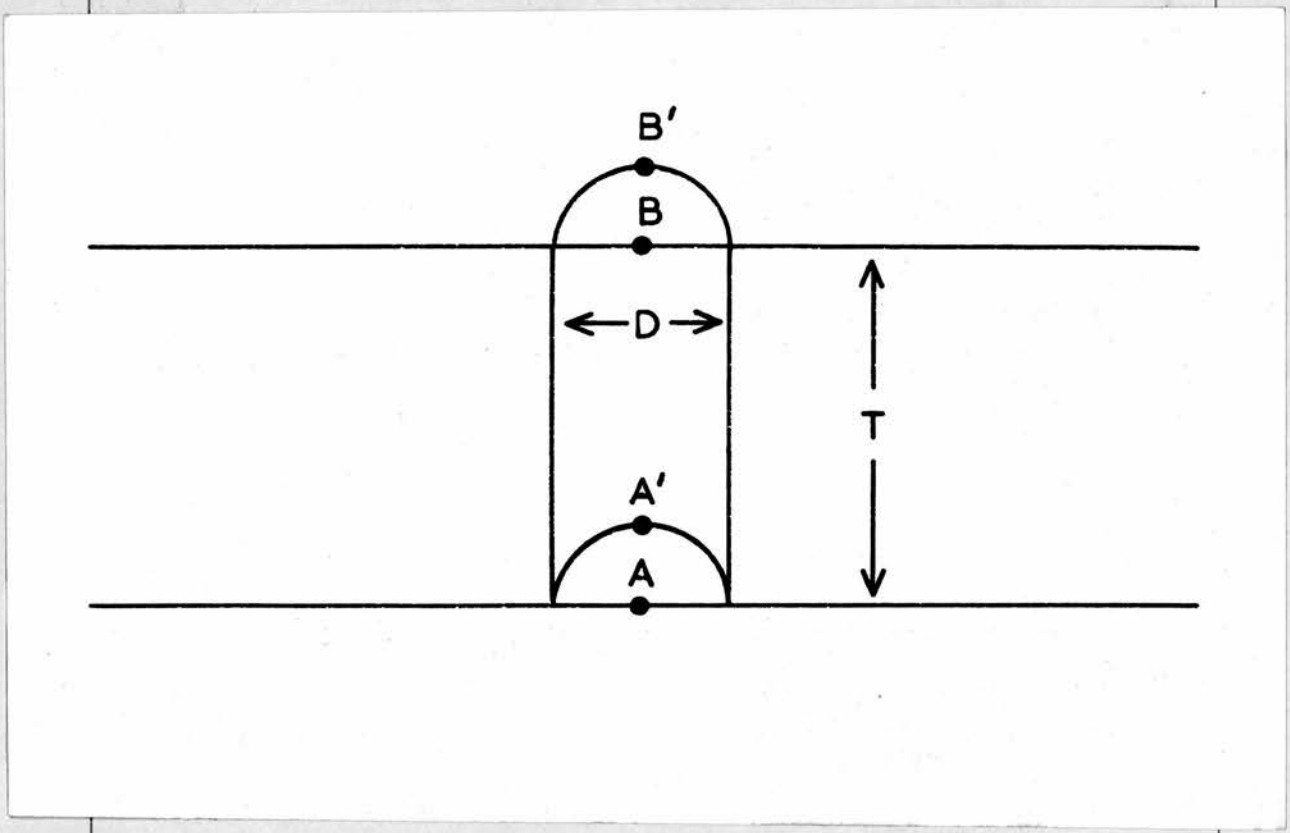
The formulae normally used to estimate volume ratio of components are only strictly accurate when applied to vanishingly thin sections. In practice (see Fig. 22) the sections have a finite thickness and what is seen in the microscope is the projection of the discrete objects, nuclei for example. As the thickness increases, screening of one object by another further complicates the issue. It would be useful if the volume ratio (R) could be determined directly from the data obtained from a "thick" section and an effort has been made to do this.

If we are looking at a section, the information which we can derive from it by any of the methods previously described is essentially a statistical estimate of the fraction of the section which/

Fig. 23

The Estimation of the relevant volume of a component from a thick tissue section by superimposition of random points over the projected image of the section.
See text for explanation of diagram.

Fig. 23



The Estimation of the relative volume of a component from a thick tissue section by superimposition of random points over the projected image of the section.
See text for explanation of diagram.

which is covered by the projection of the discrete particles, or alternatively the fraction which is free of objects or their projections.

We assume a random distribution of spheres each of diameter D. The volume ratio is taken to be R. We want to estimate the probability, f_o, that a line passing perpendicularly through the section, will not intersect any of the spheres. If we refer to Fig. 23 we can see that the probability of the line AB passing through the section is the product of two probabilities, which we assume to be independent; (a) that the point A lies on a part of the rim of the section not on or inside a sphere and (b) the probability that the volume of the cylinder of diameter D with AB as axis is not occupied by the centre of a sphere. This cylinder has a hemisphere removed from the bottom and a hemisphere added at the top and its volume is $\frac{\pi}{4} D^2T$. The fact that A does not lie inside a sphere means that there can be no sphere centre within a radium $\frac{D}{2}$, of A and for this reason we "excavate" a hemisphere from the bottom of the cylinder. At the top of the cylinder we must include the extra hemisphere because any sphere, with a centre within this hemisphere, will cut the axis AB. Points A' and B' lie on the intercept of the axis AB with the bottom and top hemispheres respectively.

Consider a point S distant r from A' along the axis AB. Let P(r) be the probability that there is no sphere centre within the cylindrical/

cylindrical volume between A' and S. Let $P(\delta r)$ be the probability that there is no sphere centre within the cylindrical volume between r and $(r + \delta r)$, where δr is a small increment of r along the axis AB. Again assuming the independence of probabilities we can write

$$P(r + \delta r) = P(r) \cdot P(\delta r) \qquad (9)$$

If the volume, δV, of cylinder between r and $(r + \delta r)$ is small then $n_o \delta V$ is the probability of at least one sphere centre lying within this volume where n_o is the mean number of centres per unit volume. The value of n_o is given in terms of R, the volume ratio, by the relation

$$n_o = \frac{6R}{\pi D^3} \qquad \text{since in unit volume the sphere volume} = R = n_o \cdot \frac{4\pi}{3} \cdot \left(\frac{D}{2}\right)^3 \qquad (10)$$

Hence $P(r + \delta r) = P(r) \cdot (1 - n_o \delta V)$

$$= P(r) \cdot \left(1 - \frac{6R}{\pi D^3} \delta V\right) = P(r) \cdot \left(1 - \frac{3}{2} \frac{R}{D} \delta r\right) \qquad (11)$$

$$\therefore \frac{dP(r)}{dr} = -\frac{3}{2} \cdot \frac{R}{D} \cdot P(r) \qquad (12)$$

Integrating

$$P(r) = e^{-\frac{3}{2} \cdot \frac{R}{D} \cdot r} \qquad (13)$$

since $P(o) = 1$ i.e. when $r = o$ the probability of there being no sphere centre between A' and S = 1. This expression gives the probability (b) mentioned above. We must now find the probability (a). This is the probability that A does not lie on or inside a sphere and is given simply by $(1 - R)$. Hence the total probability that the line passes across the section without cutting a sphere (f_o), is/

is the product of probabilities (a) and (b).

Hence, for a section of thickness T we get

$$fo = (1-R)\, e^{-\frac{3}{2}\cdot\frac{RT}{D}} \qquad (14)$$

or $$1-Ro = (1-R)\, e^{-\frac{3}{2}\cdot\frac{RT}{D}} \qquad (15)$$

where Ro is the probability that the line does cut through a sphere.

or $$R = \frac{2RoD}{3T+2D} \qquad (16)$$

This result should be compared to the formula stated (without proof) by Eränkö (1955) which does not take account of screening of one particle by another, and consequently can only be applied to infinitely thin sections which strictly speaking cannot be obtained.

Clearly the derived expression is not entirely adequate in dealing with thick sections and large R. This is because the assumption of a Poisson distribution is more valid with smaller values of R, i.e. with large R the distribution will depart from the Poisson because of geometrical constraint brought about by the finite size and solid nature of the components.

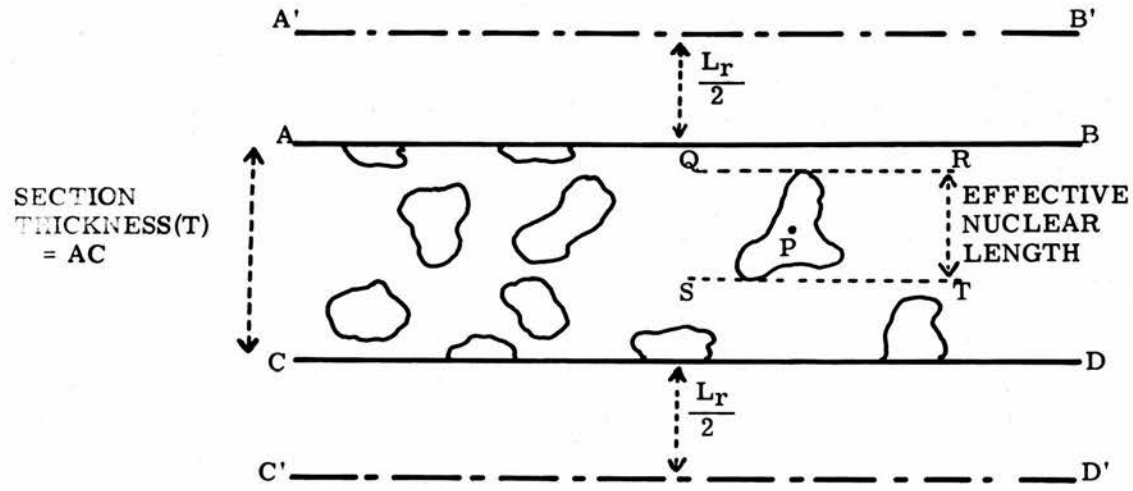
Proof E: Tissue Particle (e.g. Nuclear) Counting

Fig. 24 is a two-dimensional projection of the nuclei in a volume of tissue on a plane perpendicular to a section of tissue bounded/

Fig. 24

Estimation of the correct number of tissue components per unit volume from a microtome section.
See text for explanation of diagram.

Fig. 24



Estimation of the correct number of tissue components per unit volume from a microtome section.
See text for explanation of diagram.

bounded by AB and CD. It is obvious that some nuclei would be cut through by the microtome and counted as belonging to the section even if only a small part belonged to the section. It is desired to find a correction for this effect in the case where there is a distribution of sizes and shapes of nuclei and a random orientation of nuclei. We shall associate each nucleus with a point, so that there is a one-to-one correspondence between points and nuclei. Since the nuclei are randomly distributed, the points will be randomly distributed. For each nucleus a point P is chosen, lying in the nucleus and equidistant from two limiting tangential planes to the nucleus, QR and ST, which are parallel to the planes AB and CD of the section. The distance between the tangential planes is the effective length of the nucleus perpendicular to the section. Because of the spread of nuclear sizes and orientation, there will be a spread of effective lengths and those lengths will also be randomly distributed with respect to the planes AB and CD.

Consider all these nuclei whose effective lengths are within the limits $L_r + \delta$. If we take δ small enough we can consider each member of this sub-population to have the length L_r. If we draw two lines A'B' and C'D' each distant $\frac{L_r}{2}$ from AB and CD, respectively, then the nuclei corresponding to all points P situated between A'B' and C'D' will be counted as belonging to the section, i.e. <u>as if</u> they lay between AB and CD. If this number is N'_r then the number of/

of points actually within the section (N_r) will be given by the equation

$$N_r = N_{r'} \cdot \frac{AC}{A'C'} = N_{r'} \cdot \frac{T}{T + L_r} \quad \text{where } T = \text{section thickness (AC)}$$

$$\text{Therefore} \quad N_{r'} = N_r \cdot \frac{T + L_r}{T} \tag{16}$$

This can be repeated for each sub-population and the final result obtained by summation. Hence

$$\Sigma_r N_r = \Sigma_r\left(N_r \cdot \frac{T + L_r}{T}\right) = \Sigma_r N_r + \frac{1}{T}\Sigma_r N_r \cdot L_r \tag{17}$$

Now the mean effective length for the whole population of nuclei (L_m) is given by

$$L_m = \frac{\Sigma_r(N_r \cdot L_r)}{\Sigma_r N_r} \tag{18}$$

and from (16) and (17) we obtain

$$\Sigma_r(N'_r) = \Sigma_r(N_r)\left(1 + \frac{L_m}{T}\right) \tag{19}$$

$$\text{or} \quad \Sigma_r N_r = \Sigma_r(N'_r) \cdot \frac{T}{T + L_m} \tag{20}$$

In the case of a random distribution of equal spheres L_m is simply the diameter of the spheres. In any other case L_m is the mean nuclear length. This is obtained by measuring the length of many nuclei in any one direction, e.g. from left to right across a microscope field with the delimiting tangents (see Fig. 24) always perpendicular to the direction of scan. Assuming a random distribution/

distribution and orientation of nuclei this procedure gives the mean nuclear length perpendicular to the plane of the section.
Equation (6) gives the corrected number of points in terms of the number of nuclei counted, the section thickness, and the mean effective length of the nuclei. The volume containing this number of points is equal to the product of the section thickness (T) and the area (A) delineated by the eyepiece graticule, under which the nuclei are counted. Hence one can calculate the number of points ($\equiv$ nuclei) in the whole organ.

$$\text{i.e.} \quad N_t = \Sigma_r N_r \cdot \frac{V}{T.A} \qquad (21)$$

where N_t is the total number of nuclei in the whole organ and V is the volume of the organ. $\Sigma_r N_r$' T and A as before.

Proof F: Random line method for estimating surface/volume ratios

It is required to prove that $rh/c = \bar{d} = \frac{4V}{S}$ where r is the length of a line thrown down randomly many times over many randomly cut facets of a solid figure, h is the total number of "hits" scored by the ends of the line on the cut facets, c is the number of times the perimeter of the facets is cut by the line, and $\bar{d}$ is the mean chord intercepted; S is the surface area and V the volume of the solid figure.

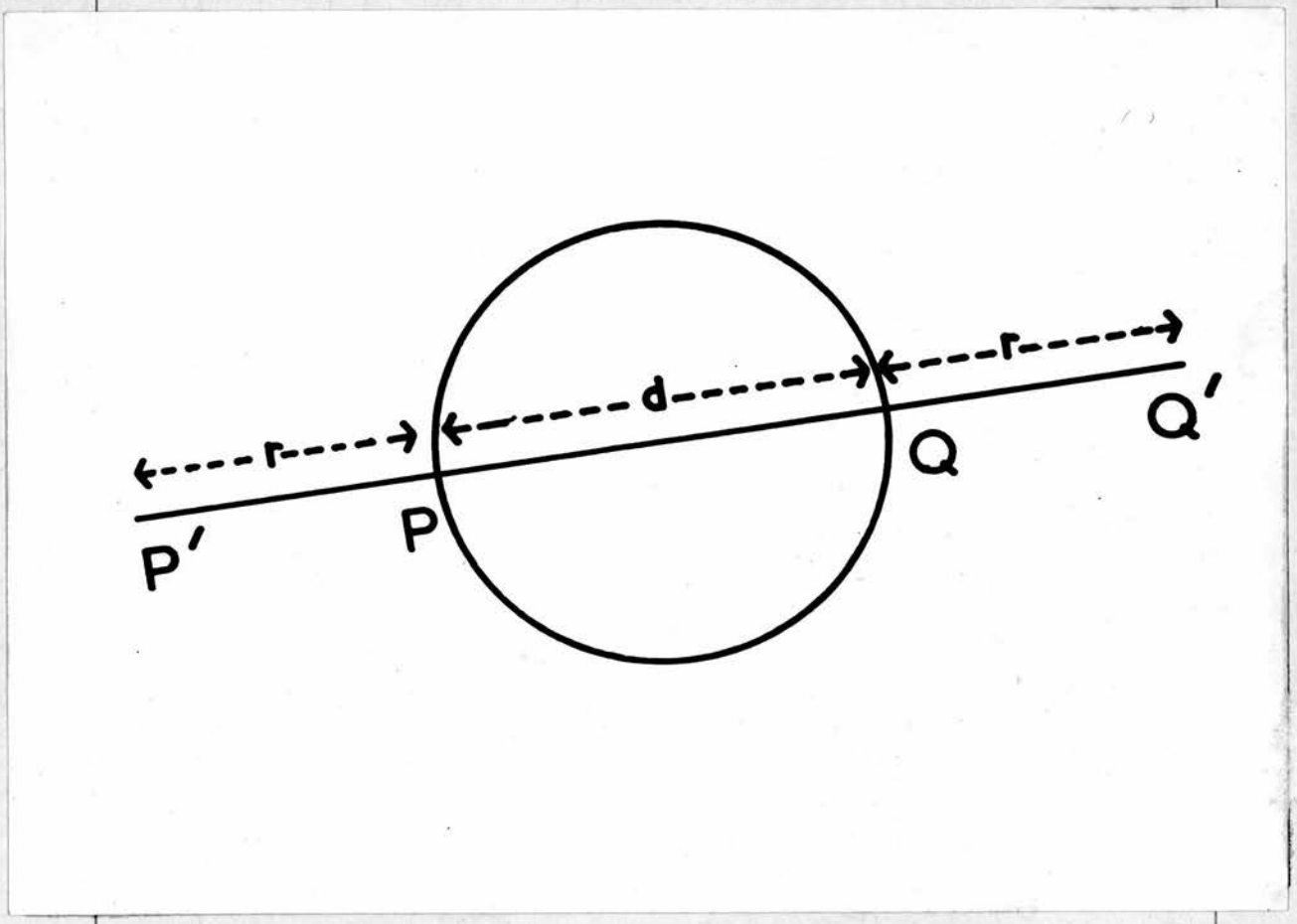
Consider any random straight line which cuts a solid figure in two points, P and Q and let PQ = d (Fig. 25).

Mark/

Fig. 25

Determination of the volume/surface area ratio of a body by the superimposition of random lines over randomly cut facets of the body. See text for explanation of diagram.

Fig. 25



Determination of the volume/surface area ratio of a body by the superimposition of random lines over randomly cut facets of the body. See text for explanation of diagram.

Mark two points, P' and Q' on the line PQ produced such that PP' = QQ' = r = the length of the line considered in Cornfield and Chalkley's formula (1951). Since the positions of the line r in space are purely random it follows that these positions which lie on P'Q' will also be random and therefore any position along P'Q' is as likely as any other. Now consider the line r, with its left-hand end initially coinciding with P' to move uniformly along P'Q' until its right-hand end coincides with Q'. Positions of this line outside this range do not contribute cuts or hits to Chalkley's equation. Considering "cuts", we can see that while the left-hand end of the line moves from P' to P, and while the right-hand end moves from Q to Q', the perimeter of the facet will be cut. Considering "hits" we can see that while either end of the line is moving from P to Q "hits" will be registered.

Hence summing for all possible positions of the line r with respect to the facet we obtain

$$\frac{\Sigma \text{ hits}}{\Sigma \text{ cuts}} = \frac{\Sigma 2d}{\Sigma 2r} = \frac{\Sigma d}{\Sigma r} \qquad (22)$$

Now if we consider a very large number n, of randomly selected lines r we have

$$\bar{d} = \text{mean chord} = \lim \frac{\Sigma d}{n} \qquad (23)$$

$$\text{and } \frac{\Sigma d}{\Sigma r} \simeq \frac{n\bar{d}}{nr} = \frac{\bar{d}}{r} \qquad (24)$$

The/

The approximation tending to equality when $n \longrightarrow \infty$

Hence $$\frac{h}{c} = \frac{\Sigma \text{ hits}}{\Sigma \text{ cuts}} = \frac{\bar{d}}{r}$$

or mean chord $= \bar{d} = \frac{rh}{c}$ (25)

but $$\bar{d} = \frac{V}{\bar{A}} = \frac{4V}{S} \quad \text{(from Cauchy's theorem)}$$

where $\bar{A}$ = mean projected area of the solid

and S = surface area of solid

Therefore $\frac{rh}{c} = \frac{4V}{S}$ which is Cornfield and Chalkley's formula (26)

From the nature of the proof it can be seen that the result also applies to re-entrant solid bodies.

SUMMARY

The theory and practice of counting and volume and surface area determinations in quantitative histology are critically examined. In an attempt to place these on a sounder footing mathematical proofs of some relevant theorems are presented. These include:-

(A) The general validity of the correction factor $\frac{T}{L + T}$ which relates the correct count of a component (mean diameter L) to the observed count in a section of thickness T.

(B) If a straight line is drawn through a volume of tissue (V) containing a random distribution of a component then $\frac{l}{L} = \frac{v}{V}$ where v is the total volume of that component, L is the length of line within the tissue and l is the sum of the intercepts of the components on the line.

(C) If a random plane of area A is drawn through a tissue and a total area of the plane (a) lies with a component then $\frac{a}{A} = \frac{v}{V}$ (symbols as in B).

(D) If a number of points are distributed randomly throughout a known volume (V) then the fraction of points coinciding with any one component = $\frac{v}{V}$ (symbols as in B).

(E) For a thick section the relative projected area of a component is not proportional to its relative volume (R) as has previously been assumed. We have derived a correction formula which describes the situation more adequately,

i.e./

$$\text{i.e. } f_o = (1 - R)e^{-\frac{3}{2}\frac{TR}{D}}$$

where f_o is the fraction of random points not lying over the component under consideration, R is the relative volume of that component and D its mean diameter. T is the section thickness. This correction formula is not very satisfactory for large values of R or T.

(F) The validity of the formula relating the surface area (S) of a component to its volume (V), i.e. $\frac{4V}{S} = \frac{rh}{c}$ where r is the length of a line thrown many times at random over many randomly cut facets of a three-dimensional figure, L is the number of hits scored by the ends of the line over the cut area, and c the number of times the line cuts the surface of the figure.

The limitations and usefulness of the various practical techniques are discussed.

APPENDICES

APPENDIX TABLE 1 - Experiment P

Changes in Gland Weight with Methylthiouracil (M.T.) Administration

Days on M.T.	Gland Weight (mg.)	Days on M.T.	Gland Weight (mg.)	Days on M.T.	Gland Weight (mg.)
0	16.8 24.4 20.0 17.3 17.5	15	65.8 79.8 75.8 53.2 60.2	30	61.2 70.9 76.2 79.0 53.2
3	17.0 20.3 17.7 12.3 15.7	18	66.4 65.4 58.4 93.0 60.0	33	60.8 54.0 75.2 82.4 110.1
6	33.2 26.8 32.2 32.8 37.6	21	53.2 58.8 60.2 78.2 70.6	36	53.5 61.8 92.8 101.4 71.4
9	41.8 39.0 38.6 39.2 35.4	24	81.4 75.9 69.0 49.6 47.2		
12	67.6 83.8 52.8 53.8 60.8	27	57.0 108.6 71.6 68.2 99.0		

APPENDIX TABLE 2 - Experiment A (Controls)

Effects of Methylthiouracil (M.T.) on the Rat Thyroid Gland Weight, Mean Follicular Cell Concentration and Mean Follicular Cell Volume.

Days on M.T.	Gland Weight (G.W.) (mg.)	Mean Follicular Cell Volume (M.C.V.) μ^3	Follicular Cell Concentration (F.C.C.) $10^6/10mm.^3$	Days on M.T.	G.W. mg.	M.C.V. μ^3	F.C.C. $10^6/10mm.^3$
0	21.3	1118	3.39	4	18.1	898	4.00
	12.3	1090	3.88		12.8	852	4.92
	18.6	1324	3.47		16.0	1027	3.99
	20.9	1060	4.05		16.6	1219	3.52
	15.8	1085	3.13		12.0	928	4.32
8	18.2	1154	3.25	12	12.8	914	4.26
	12.2	986	3.95		16.0	1170	3.50
	15.7	892	3.58		19.9	940	4.35
	15.3	1023	4.00		22.6	1190	3.44
	13.8	1295	3.47		17.6	1161	3.61
16	17.4	1538	2.99	20	20.8	1053	3.70
	17.0	892	3.69		20.5	910	3.40
	13.0	990	4.03		16.9	1223	3.51
	19.8	1297	3.31		16.2	1091	3.66
	14.8	851	4.81		15.6	1085	4.05

APPENDIX TABLE 3 - Experiment A (Goitrogenic Response)

Effects of Methylthiouracil on the Rat Thyroid Gland Weight, Mean Follicular Cell Concentration and Mean Follicular Cell Volume

Days on M.T.	G.W. (mg.)	M.C.V. μ^3	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	M.C.V. μ^3	F.C.C. $10^6/10mm.^3$
0	16.3	863	3.59	12	37.3	1703	3.58
	16.2	1275	3.53		53.0	1535	2.87
	19.6	804	3.89		36.8	1528	3.53
	10.4	1437	3.41		49.5	1622	3.39
	13.4	1065	4.13		44.4	1585	3.27
2	16.0	1332	3.30	14	42.1	2007	3.04
	18.4	1146	3.83		56.5	1734	3.17
	10.6	871	4.81		40.8	2074	3.13
	18.6	1158	4.05		58.5	1850	3.13
	12.4	1023	4.00		47.4	1323	4.60
4	10.4	1513	3.96	16	40.2	1821	3.57
	11.2	1301	3.22		46.8	1331	4.20
	20.8	1667	3.95		61.6	1596	3.82
	19.6	1143	4.19		41.0	1812	3.42
	19.7	1445	3.87		62.3	1673	3.34
6	29.2	1861	3.27	18	42.0	1482	3.37
	23.6	1712	3.21		41.2	1610	3.35
	31.9	1174	4.08		43.6	1700	3.29
	24.3	1535	3.25		41.2	1640	3.65
	25.1	1319	3.49		41.1	2060	2.96
8	22.2	2021	3.36	20	39.9	1902	3.10
	36.0	1060	3.67		31.2	1824	3.45
	27.4	1281	4.52		61.6	1833	3.27
	25.5	1107	3.34		69.8	1762	3.00
	34.4	996	3.61		62.7	1688	3.49
10	36.8	1682	3.32	22	55.7	1605	3.98
	19.6	1191	3.94		55.5	1441	4.02
	39.0	1430	3.70		76.0	1740	3.21
	46.0	1297	3.68		42.7	1517	3.29
	38.6	1411	3.48		58.7	1555	3.60

APPENDIX TABLE 4 - Experiment G - (0 rads)

Effects of Methylthiouracil on the Rat Thyroid Gland Weight, Mean Follicular Cell Concentration and Mean Follicular Cell Volume

Days on M.T.	G.W. (mg.)	M.C.V. μ^3	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	M.C.V. μ^3	F.C.C. $10^6/10mm.^3$
0	15.0	1152	3.23	8	24.4	1764	2.76
	14.2	907	3.61		32.2	1543	4.11
	10.2	1189	4.72		30.9	1595	3.92
	16.0	1367	3.54		30.3	1591	2.97
	8.0	1198	3.27		39.8	1773	2.93
	12.1	1381	3.61		35.3	1439	3.56
	9.9	1380	3.58		34.8	1449	3.25
	10.0	925	3.91	10	36.5	1533	3.62
	13.3	1138	3.20		37.2	1411	4.41
	12.4	1108	3.27		35.8	1521	3.56
	13.1	978	3.82		43.5	1270	4.63
	13.8	835	3.53		33.0	1529	3.62
	16.0	1007	3.78		34.4	1787	3.36
	14.0	1056	4.45		43.0	2019	3.13
2	11.4	851	4.05	12	48.5	1915	3.68
	12.7	982	4.21		43.6	2118	3.06
	14.1	923	3.37		47.0	1677	3.43
	16.1	1283	3.77		33.5	1570	3.52
	15.9	1085	3.61		38.4	1757	3.52
	13.2	825	3.22		40.4	2318	3.00
	12.0	991	3.95		37.2	2131	3.05
4	17.3	1240	2.90	14	60.6	1704	3.86
	17.1	1140	4.29		38.8	1661	3.03
	23.8	1308	3.71		52.0	1404	3.83
	15.8	1112	2.91		54.3	1540	3.92
	15.6	1119	3.29		36.4	1814	2.76
	15.4	1205	3.21		45.1	1516	3.56
	15.0	1221	3.72		70.2	2069	3.84
6	22.0	1737	3.52	16	58.3	2028	3.46
	21.4	1766	3.52		53.6	1773	4.17
	20.9	1714	4.20		42.6	1860	3.20
	30.8	1733	3.40		40.2	1916	3.53
	28.8	1850	3.37		49.9	1980	3.62
	24.1	1651	3.49		45.8	1751	2.79
	24.0	2114	3.77		52.7	2295	3.23

APPENDIX TABLE 5 - Experiment G (200 rads)

Effect of X-irradiation on the Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration.

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	16.2	3.31	12	43.0	4.11
	11.8	3.01		42.6	3.68
	12.6	4.34		36.8	3.10
	13.3	3.89		23.7	4.32
	12.1	3.51		34.8	3.36
	12.0	4.46		31.3	3.51
	11.2	4.51		27.7	3.35
	10.8	3.59			
	8.1	4.17			
	13.0	3.96			
	11.4	3.25			
	14.9	3.52			
	12.1	2.95			
	7.7	4.37			
2	15.2	3.07	14	41.6	3.60
	12.4	3.26		30.5	3.69
	11.0	4.61		38.7	3.82
	18.6	3.51		34.0	3.43
	11.2	3.52		41.5	4.11
	11.1	3.08		41.4	3.66
	12.6	3.85		41.6	3.75
4	16.0	3.45	16	42.4	4.03
	20.7	2.78		32.4	3.98
	18.6	2.85		46.7	3.63
	11.7	4.07		34.1	3.89
	19.7	3.22		42.0	3.25
	19.6	3.34		36.8	3.62
	11.4	3.95		37.4	3.45
6	25.1	3.28	18	46.2	3.93
	25.2	3.09		56.4	3.89
	23.1	3.78		47.0	3.17
	21.2	3.62		51.5	3.03
	24.5	2.91		37.7	3.89
	23.6	2.48		71.6	3.20
	19.7	5.13		40.8	2.93
8	30.5	3.65	20	33.4	3.63
	29.8	3.22		36.3	3.62
	26.4	3.19		31.6	3.72
	34.8	3.38		41.8	3.85
	20.7	3.75		37.7	3.49
	23.5	2.88		53.4	3.97
	22.3	2.61		45.0	3.98
10	29.2	3.54	22	56.6	3.63
	39.3	3.57		53.2	3.62
	29.8	3.14		43.2	3.84
	29.5	2.96		71.2	3.61
	32.7	3.73		61.6	2.71
	47.2	3.64		37.6	4.20
	28.5	3.71		43.1	4.17

APPENDIX TABLE 6 - Experiment G (300 rads)

Effect of X-Irradiation on the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	Days on M.T.	G.W. (mg.)
0	14.1 16.4 13.0 16.0 14.4 12.8 14.2 13.7 14.2 12.1 14.0 15.4 15.4 12.0 12.5 13.9	8	24.4 22.6 23.5 28.2 33.7 26.9
		9	31.6 32.5 28.5 30.8 35.8 34.8 27.1
2	19.2 15.0 15.2 14.4 11.8 14.2 11.4	11	29.2 27.2 36.3 30.6 30.0
4	22.8 23.6 19.3 22.0 17.2 20.4 20.1	13	33.1 37.5 28.3 47.0 20.4 38.3 34.5
6	26.0 20.3 20.3 24.1 28.6 20.4 27.2	15	42.0 48.0 42.0 49.4 49.2 45.1 38.1

APPENDIX TABLE 6 - Experiment G (300 rads) continued

Days on M.T.	G.W. (mg.)	Days on M.T.	G.W. (mg.)
17	37.8 46.8 45.4 48.0 48.8 39.8	25	59.4 69.0 46.0 66.0 37.0 52.6
19	47.3 30.6 39.0 37.2 47.2 47.2 32.4 49.2	27	56.3 52.7 64.5 42.4 45.0 42.9 58.0
21	34.2 34.0 39.5 39.2 40.3 42.2 47.2	29	57.0 53.0 38.4 49.0 68.0 52.1 39.8
23	40.8 54.6 56.4 48.4 29.1 56.8 30.2		

APPENDIX TABLE 7 - Experiment G (400 rads)

Effect of X-irradiation on the Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	6.1	5.42	12	36.4	3.08
	10.4	3.87		32.9	4.43
	10.9	3.39		32.3	3.66
	20.5	4.09		34.4	3.52
	14.2	3.35		37.0	3.35
	15.4	3.23		32.4	3.76
	13.4	3.75		29.2	4.06
	12.2	5.06			
	11.0	3.46			
	10.2	4.70			
	15.0	4.17			
	12.8	5.21			
	18.3	3.75			
	11.4	3.24			
2	15.5	3.99	14	41.0	2.99
	11.7	4.15		37.5	3.22
	11.0	3.67		38.9	3.32
	15.6	3.17		48.5	3.69
	11.4	4.21		35.6	3.38
	13.3	4.55		67.0	2.67
	10.4	3.78		33.7	2.83
4	15.7	3.19	16	40.0	3.64
	18.2	3.04		30.0	4.47
	20.5	3.38		40.4	4.08
	16.6	3.47		27.2	3.56
	11.2	3.95		33.3	3.60
	18.8	3.62		43.9	3.94
	15.8	3.47		25.2	3.99
6	29.7	3.62	18	43.1	3.61
	24.0	5.62		41.4	3.37
	26.2	3.48		27.5	4.68
	22.5	3.34		36.2	3.74
	27.4	3.02		30.4	3.78
	24.0	3.73		32.7	3.82
	30.2	2.72		38.9	3.67
8	39.7	3.59	20	46.2	3.71
	30.1	3.77		40.0	3.50
	19.8	3.26		36.2	3.72
	34.5	3.15		51.2	3.33
	29.7	3.45		31.0	3.33
	27.8	2.93		45.8	4.24
	20.1	3.20		26.0	3.44
10	35.4	3.44	22	38.2	3.71
	22.6	3.48		30.8	4.02
	30.8	3.28		40.3	3.96
	30.6	3.65		36.6	3.26
	41.9	3.61		32.0	4.21
	23.5	3.07		33.6	3.51
	25.5	4.13		41.2	4.10

APPENDIX TABLE 7 - Experiment G (400 rads) continued

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
24	54.3	3.56	37	54.4	4.01
	50.8	3.49		33.1	4.02
	57.9	3.73		67.7	3.05
	59.6	3.22		42.2	3.73
	31.4	4.26		37.1	3.23
	42.0	3.64		39.1	3.45
	38.6	4.37		50.0	3.40
26	43.3	3.18	39	48.7	2.78
	48.0	3.96		42.6	3.82
	53.6	3.52		35.8	4.30
	53.8	3.31		45.9	3.76
	44.0	3.67		34.2	3.61
	49.2	3.11		39.6	3.45
	40.2	3.73		40.2	3.83
28	64.4	3.23	41	61.5	3.61
	43.0	3.85		69.2	3.79
	24.1	3.51		38.4	3.73
	43.0	3.62		47.0	3.76
	55.9	3.23		50.4	3.98
	34.1	3.78		61.2	3.35
	63.9	3.13		37.0	3.49
30	30.8	4.02	43	60.5	3.57
	41.5	3.32		37.6	3.14
	55.7	3.80		62.6	3.39
	52.0	3.65		67.8	4.00
	39.1	3.73		49.0	3.49
	52.7	3.74		45.2	3.68
	45.6	3.20		51.7	3.90
32	52.8	3.61	45	57.6	3.52
	64.8	3.27		55.4	3.74
	33.6	3.64		12.0	3.38
	52.2	2.83		36.0	3.28
	41.3	3.68		54.7	3.00
	41.4	3.73		41.1	3.46
	died	3.62			
35	60.3	3.24	47	65.0	3.50
	58.1	3.23		49.4	3.29
	42.0	3.16		42.2	3.35
	46.0	4.05		57.0	3.10
	43.0	3.48		55.0	3.25
	38.5	3.38		43.0	3.43
	46.0	3.13			

APPENDIX TABLE 8 - Experiment H (0 rads)

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	F.C.C. 10^6/10mm.^{3}	Days on M.T.	G.W. (mg.)	F.C.C. 10^6/10mm.^{3}
0	13.2	3.53	8	45.8	
	12.8	5.43		37.6	
	12.8	4.72		35.6	
	12.1	4.85		35.1	
	10.9	4.33		40.0	
	14.6	4.63		31.8	
	10.7	3.77		47.4	
	9.6	4.37	10	37.2	3.80
	14.4	4.55		43.4	3.37
	17.0	4.20		52.2	3.40
	14.7	3.62		43.6	4.02
	11.1	5.58		35.0	3.71
	16.2	4.29		35.6	3.40
	14.0	4.04		38.6	3.81
2	13.2	3.65	12	47.8	
	12.2	3.65		46.9	
	11.2	3.65		39.6	
	9.1	4.04		44.2	
	12.3	4.11		34.6	
	11.7	3.95		37.8	
	16.1	3.71		39.2	
4	16.1	3.71	14	48.2	4.78
	18.7	3.28		53.2	3.95
	17.6	3.59		53.9	3.77
	17.4	3.22		66.8	3.31
	15.3	2.91		43.2	4.02
	15.9	3.13		44.0	3.28
	17.0	2.94		56.6	3.31
6	36.4	3.06	16	35.6	
	26.6	3.65		50.8	
	29.0	3.49		44.8	
	26.8	3.74		36.7	
	22.9	3.16		29.4	
	25.2	3.16		59.6	
	18.0	4.11		45.4	
				51.0	

APPENDIX TABLE 9 - Experiment H (600 rads)

Effects of X-Irradiation on the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	Days on M.T.	G.W. (mg.)
0	15.2	17	37.8
	15.1		39.9
	10.1		27.0
	10.6		29.5
	13.2		31.4
	14.6		43.9
	15.7		29.7
	9.7	20	40.0
	11.2		40.1
	11.2		50.4
	13.2		33.0
	9.5		48.0
	14.2		31.4
	12.5		41.4
2	10.8	25	39.9
	12.6		35.1
	11.2		50.8
	10.4		30.6
	12.2		39.3
	16.2		46.6
	11.6		64.8
4	14.3	28	34.1
	16.2		40.1
	8.6		40.6
	15.2		41.7
	14.8		52.2
	17.5		37.0
	11.4		42.5
6	18.4	32	47.4
	38.0		42.8
	27.0		52.2
	21.6		45.4
	37.7		32.2
	31.6		32.4
	25.0		47.6
8	38.0	35	31.9
	25.6		37.2
	29.0		43.9
	29.6		17.9
	27.7		49.4
	30.3		43.8
	29.5		47.3
10	37.7	39	25.6
	43.4		47.2
	31.7		41.4
	26.1		47.4
	29.5		61.3
	43.5		45.8
	27.7		54.4
14	38.1	42	43.5
	34.7		51.1
	56.2		34.2
	41.3		42.4
	36.0		50.3
	41.7		44.9
	39.0		

APPENDIX TABLE 10 - Experiment H (800 rads)

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	11.9	3.49	8	29.4	
	7.4	4.49		21.4	
	11.7	3.33		28.1	
	9.8	4.86		27.7	
	13.2	3.64		25.0	
	12.2	4.22		29.2	
	9.9	4.86		30.0	
	8.2	4.37	10	34.7	
	8.4	4.71		26.4	
	12.5	4.03		25.7	
	9.8	4.52		35.6	
	10.8	3.88		35.2	
	10.5	3.94		31.4	
	9.7	3.37		28.7	
2	18.6	3.71	14	35.0	3.40
	11.2	4.21		32.4	3.81
	16.8	4.14		30.4	3.67
	16.1	3.59		41.9	4.01
	9.1	3.49		29.8	3.56
	11.8	3.59		27.2	3.75
	14.9	3.81		36.5	3.80
4	18.2	3.81	18	33.6	
	15.6	3.56		31.0	
	17.4	3.53		40.8	
	17.9	3.34		35.6	
	14.5	3.49		28.9	
	17.8	3.41		30.0	
	14.1	3.56		43.8	
6	25.4	3.65	21	34.8	
	22.3	3.89		32.2	
	28.9	3.91		46.2	
	26.8	3.25		31.0	
	27.0	3.10		49.8	
	22.2	3.86		31.1	
	30.5	3.75		38.2	

APPENDIX TABLE 10 - Experiment H (800 rads) continued

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
25	34.8	3.74	39	56.8	3.10
	21.1	3.76		31.2	3.25
	45.4	6.15		34.0	3.40
	32.0	3.47		25.2	3.40
	27.2	3.41		28.9	3.76
	33.8	3.63		32.7	3.01
	34.6	3.90		26.7	3.95
28	50.5		42	44.1	3.65
	39.8			37.6	4.00
	30.8			47.5	3.95
	37.2			42.2	3.76
	31.9			29.1	3.63
	28.8			39.0	3.63
	22.4			48.6	3.89
32	31.8		46	24.0	3.74
	32.2			37.6	3.25
	30.6			42.2	3.10
	39.8			56.5	3.00
	29.0			46.0	3.76
	36.0			41.9	3.71
	38.8			29.3	3.10
35	30.4	3.75	49	41.0	3.25
	27.8	3.80		37.8	4.71
	45.0	4.10		46.9	3.59
	27.7	3.50		43.1	3.49
	29.0	3.40		35.9	3.25
	49.0	3.56		61.2	3.74
	33.6	3.89		38.2	3.90

APPENDIX TABLE 11 - Experiment H (1000 rads)

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	8.8	4.01	8	20.1	2.58
	8.9	3.60		21.4	3.00
	9.8	5.05		25.0	3.66
	12.8	3.03		22.0	3.13
	10.8	2.92		24.2	2.99
	8.1	3.11		42.0	3.06
	9.1	4.08		21.0	2.99
	10.0	4.84	10	22.9	
	11.0	4.54		26.9	
	8.7	4.21		16.6	
	14.9	4.12		21.2	
	13.7	4.30		22.8	
	9.3	3.91		17.6	
	14.3	4.21		27.2	
2	8.7	3.86	12	17.8	3.40
	13.0	3.14		35.3	3.31
	17.2	3.34		30.6	3.65
	10.1	3.18		30.2	2.64
	15.8	3.52		33.8	3.40
	9.4	3.30		23.4	3.92
	12.5	3.98		dead	
4	10.8	4.28	16	29.4	
	18.1	3.25		29.0	
	12.2	3.48		32.0	
	12.3	3.17		36.6	
	12.4	3.40		26.2	
	11.7	4.17		33.0	
	7.6	5.21		31.0	
6	29.0		20	34.5	
	20.0			20.9	
	18.0			30.9	
	17.0			29.2	
	21.4			20.9	
	18.0			31.8	
	15.0			28.1	

APPENDIX TABLE 11 - Experiment H (1000 rads) continued

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
23	30.5 35.4 30.3 25.2 28.0 28.9 26.9	3.12 3.43 3.86 3.74 3.47 3.04 3.43	37	40.2 24.8 24.9 26.2 33.4 29.8 40.7	
27	18.7 28.0 28.8 38.4 22.0 44.6 26.7		41	31.3 25.6 22.0 28.5 22.7 27.7 28.0	3.92 3.62 3.81 3.51 3.42 3.33 3.49
30	27.3 40.2 31.2 35.0 28.5 31.3 31.4		44	22.1 28.2 37.6 37.2 34.3 24.4 32.3	3.70 3.50 3.50 3.56 3.82 3.95 3.50
34	31.9 23.7 27.3 22.0 33.4 24.3 23.0	3.37 3.28 3.77 3.64 4.26 3.51 3.68	48	34.1 28.3 31.3 34.8 25.6 25.4 27.6	

APPENDIX TABLE 11 - Experiment H (1000 rads) continued

Days on M.T.	G.W. (mg.)	F.C.C. 10^6/10mm.^{3}	Days on M.T.	G.W. (mg.)	F.C.C. 10^6/10mm.^{3}
51	33.6 24.8 23.3 50.2 40.2 32.2 35.0		69	26.3 32.7 31.4 40.1 29.1 46.0 38.2	3.62 3.23 3.41 3.78 3.57 3.24 3.90
55	21.9 24.0 25.0 21.1 21.8 23.8 21.3	3.58 4.04 3.90 3.90 4.75 3.99 4.28	92	17.0 25.2 22.4 29.7 31.2	
62	24.4 24.0 25.4 23.2 21.8 28.8 22.4		132	27.5 32.2 23.0 64.3	3.58 3.73 3.46 3.26

APPENDIX TABLE 12 - Experiment J (0 rads)

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	22.0	3.75	8	43.6	3.00
	20.2	4.23		45.8	3.64
	21.9	4.09		45.2	3.71
	12.3	3.95		40.8	3.95
	19.5	4.10		27.5	4.08
	12.6	3.09		36.0	3.55
2	16.6	4.11	10	48.8	3.45
	17.1	3.55		51.2	3.86
	12.4	4.01		41.8	4.73
	19.5	3.75		48.9	3.55
	15.8	3.07		45.8	3.85
	12.4	4.32		57.0	3.00
4	31.3	3.85	12	54.8	3.40
	25.4	3.65		42.4	3.37
	24.5	3.89		46.4	4.02
	20.0	4.28		45.0	3.71
	20.0	3.05		53.0	3.45
	19.6	2.98			
6	28.4	4.01	14	53.4	4.00
	31.0	3.75		43.0	3.71
	35.2	3.89		49.4	3.28
	26.2	3.68		68.8	3.27
	35.3	4.31		50.0	4.56
	41.8	3.36		44.6	3.25

APPENDIX TABLE 13 - Experiment J (1500 rads)

Effects of X-Irradiation on the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	Days on M.T.	G.W. (mg.)
0	16.3 16.2 19.6 10.4 13.4 -	6	23.4 21.0 31.2 21.9 20.5 25.4
2	13.6 13.3 13.7 13.9 13.0 10.6	8	18.1 22.5 14.8 20.6 26.9 26.0
4	17.0 15.3 22.1 17.9 15.1 15.6	10	27.8 18.3 22.3 21.8 26.2 22.5

APPENDIX TABLE 14 - Experiment J (2500 rads)

Effects of X-Irradiation on the Mean Follicular Cell Concentration and Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) Administration

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	18.2	3.99	6	21.1	4.00
	12.2	4.21		13.7	4.35
	15.7	3.75		20.6	3.94
	15.3	4.09		23.8	3.76
	13.8	4.15		19.8	5.26
	-	-		18.2	3.88
2	18.3	4.13	8	16.6	4.00
	12.1	4.28		18.6	3.70
	16.8	4.03		15.2	4.65
	15.2	3.91		-	-
	13.7	4.43		17.0	3.64
	13.8	3.89		-	-
4	19.4	3.51	10	18.2	3.70
	19.6	4.13		33.9	3.03
	27.0	4.10		18.4	3.67
	16.8	3.79		-	-
	-	-		19.6	3.61
	24.0	3.70		20.0	3.67

APPENDIX TABLE 15 - Experiment H (0 months)

Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) At Intervals after 1000 rads X-Irradiation

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	8.8	4.01	6	29.0	
	8.9	3.60		20.0	
	9.8	5.05		18.0	
	12.8	3.03		17.0	
	10.8	2.92		21.4	
	8.1	3.11		18.0	
	9.1	4.08		15.0	
	10.0	4.84	8	20.1	2.58
	11.0	4.54		21.4	3.00
	8.7	4.21		25.0	3.66
	14.9	4.12		22.0	3.13
	13.7	4.30		24.2	2.99
	9.3	3.91		42.0	3.06
	14.3	4.21		21.0	2.94
2	8.7	3.86	10	22.9	
	13.0	3.14		26.9	
	17.2	3.34		16.6	
	10.1	3.18		21.2	
	15.8	3.52		22.8	
	9.4	3.30		17.6	
	12.5	3.98		27.2	
4	10.8	4.28	12	17.8	3.40
	18.1	3.25		35.3	3.31
	12.2	3.48		30.6	3.65
	12.3	3.17		30.2	2.64
	12.4	3.40		33.8	3.40
	11.7	4.17		23.4	3.92
	7.6	5.21		dead	

APPENDIX TABLE 16 - Experiment H (3months)

Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) At Intervals after 1000 rads X-Irradiation

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	12.7 12.1 5.4 14.4 10.0	4.22 4.36 4.34 3.36 4.01	6	22.9 17.4 16.9 15.7 22.2	3.73 4.02 2.95 3.30 3.65
2	12.4 15.4 16.7 14.6 16.9	2.81 4.75 3.76 3.44 3.41	10	22.6 15.6 22.6 19.5 -	4.77 3.46 3.73 4.29 -
4	23.8 8.1 17.2 18.2 18.6	3.09 4.62 4.29 3.81 4.65	12	18.4 17.7 17.7 21.9	3.26 5.32 4.04 4.10

APPENDIX TABLE 17 - Experiment H (6 months)

Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) At Intervals after 1000 rads X-Irradiation

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	14.3 19.8 15.7 9.1 12.7	4.25 3.71 5.12 3.58 4.10	6	17.0 16.6 21.2 24.2 27.0	3.68 4.65 2.88 4.07 3.26
2	13.9 15.4 18.6 18.5 12.1	5.17 4.02 3.63 3.74 3.96	8	19.8 21.3 24.1 15.8 20.7	4.00 4.09 3.64 3.33 3.29
4	21.1 14.3 21.8 15.6 15.7	4.47 4.26 3.91 3.91 3.15	10	17.9 18.6 17.1 23.5	4.38 4.90 3.72 3.37

APPENDIX TABLE 18 - Experiment H (9 months)

Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) At Intervals after 1000 rads X-Irradiation

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	13.2	3.11	6	20.7	3.02
	8.6	2.87		21.8	3.50
	11.4	5.23		16.3	3.77
	11.5	5.55		16.3	4.88
	12.3	4.98		21.8	3.72
2	10.8	3.80	8	16.4	3.65
	14.9	3.67		18.9	4.28
	13.6	4.09		16.7	3.76
	12.5	4.57		20.5	3.79
	11.7	3.85		18.0	3.87
4	14.5	4.28	10	15.9	3.69
	19.5	2.95		39.3	3.45
	15.3	3.43		26.0	3.11
	15.1	3.28		13.0	4.21
	19.3	3.53		22.9	2.78

APPENDIX TABLE 19 - Experiment H (12 months)

Comparison of the Goitrogenic Response of the Rat Thyroid to Methylthiouracil (M.T.) At Intervals after 1000 rads X-Irradiation

Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$	Days on M.T.	G.W. (mg.)	F.C.C. $10^6/10mm.^3$
0	14.9 29.0 7.4	4.17 3.24 3.90	10	15.3 17.8 17.2	2.98 3.54 3.86
2	9.4 15.9 12.0	5.02 3.93 3.74	12	22.3 19.5 16.3	3.75 4.65 3.80
6	14.4 13.5 14.6	3.30 4.10 4.17			

APPENDIX TABLE 20

Long Term Effect of 1000 rads X-Irradiation on the One Hour Percentage Uptake of a Tracer Dose of Radioiodine (^{131}I) in the Rat

Time After Irradiation	% Thyroid Uptake of ^{131}I	Time After Irradiation	% Thyroid Uptake of ^{131}I
0 (controls - no radiation)	13.3 16.7 16.3 18.5 15.0 9.1 17.6 15.4 20.5 20.0 19.3 15.0 13.5 13.0	22 days	16.0 23.1 12.3 13.9 15.5 17.0
		3 months	6.7 13.2 14.2 17.4 10.8 14.2
24 hours	15.0 15.6 13.7 13.5 12.7 14.9	6 months	16.2 11.4 18.4 15.8 20.5 16.5
3 days	13.0 16.0 10.9 14.8 16.5 9.5	9 months	20.4 7.1 20.2 18.2 11.7
7 days	16.1 15.1 19.1 23.5 15.7 27.0	12 months	12.9 12.4 10.7 10.7
14 days	16.3 10.3 11.7 16.0 17.2 13.1		

APPENDIX TABLE 21

Short Term Effect of X-Irradiation on the One Hour Percentage Thyroidal Uptake of a Tracer Dose of Radioiodine (^{131}I) in the Rat

Hours After Irradiation	Rad Dose					
	0	500	1000	1500	2000	2500
1	10.3 14.1 18.1 16.3 10.5 9.5 12.2 11.7 12.2 10.5 12.4 15.0	4.6 6.8 11.2 8.2 10.1 8.1	4.5 6.5 6.8 3.2 7.6 8.8	5.5 8.5 7.1 14.9 12.3 6.8	7.0 9.2 11.0 11.3 9.0 10.4	8.6 5.9 6.2 4.4 4.6 7.3
6		8.3 12.5 11.2 15.0 14.3 18.5	13.3 8.6 21.1 8.7 21.3 18.7	18.7 13.7 21.4 20.0 27.5 16.5	10.7 8.0 7.7 -	8.4 12.0 13.4 12.2 18.6 14.9
24		10.3 19.9 14.9 10.5 14.8 10.3	11.0 14.7 15.7 16.7 21.6 13.7	9.1 12.1 13.3 19.2 12.0 11.8	15.1 11.7 24.9 21.2 14.8 16.6	14.7 17.4 23.1 9.2 17.0 13.1
48		11.4 21.1 8.7 16.8 13.3 22.5	24.0 10.0 8.2 16.5 15.5 23.2	18.6 17.8 17.3 14.7 20.6 11.9	19.5 27.1 22.4 26.0 16.8 20.9	15.4 14.5 - 17.8 14.3 19.7
72		10.5 13.0 7.8 12.4 13.9 13.5	8.1 5.9 14.4 8.4 7.5 11.3	5.3 6.7 10.4 11.0 9.7 5.9	9.5 9.2 10.8 10.0 12.1 13.2	11.5 15.7 13.9 11.7 7.3 8.7
96	12.0 6.6 10.7 12.8 10.2 11.8 10.8 15.9 10.6	12.0 13.6 17.5 17.6 8.0 20.0	19.5 - 12.4 15.1 8.3 9.8	7.0 5.8 7.2 8.4 14.3 6.8	9.9 5.1 9.7 8.8 7.0	12.8 3.8 4.9 3.6 9.6

APPENDIX TABLE 22

Effect of 800 rads γ-radiation (^{60}Co) on Thyroidal Discharge of a Tracer Dose of Radioiodine (^{131}I) in 5 Thyrotoxic Patients

Days Before and After Irradiation	Counts ($\times 10^4$) per minute (Corrected for Radioactive Decay)				
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
-7		6.1			
-6		5.6	10.1		
-5	7.1	5.1	9.2		6.5
-4	6.6	4.7	8.4	16.3	5.9
-3	6.3			13.4	5.1
-2	5.9		6.8		5.0
-1	5.4	3.5		11.1 9.3	4.9 4.7
0 (Irradiation)					
+1	5.8	2.9	4.7	8.4	3.9
+2	5.0	2.5	4.4	7.0	3.9
+3	4.8	2.15		7.3	
+4	4.1				3.3
+5	3.7		2.2		2.7
+6	3.2	1.9	3.2	5.2	2.8
+7	3.0	1.9	2.9	4.8	2.7
+8			2.7	4.5	
+9			2.6	3.8	

APPENDIX TABLE 23

Effect of 800 rads γ-radiation (^{60}Co) on Thyroidal 30 minute Radioiodine (^{132}I) Uptake in 5 Thyrotoxic Patients

Days Before and After Irradiation	30 minute % Uptake of a Tracer Dose of ^{132}I				
	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
-6		25.0	12.5		17.5
-5		18.5	14.0		
-4		28.5	15.0	25.3	
-3				29.0	14.3
-2	23.7				
-1	23.5 19.0	18.0	18.7 19.0	11.0	14.0
0 (Irradiation)					
+1		18.2		16.5	
+2	30.0	19.0	19.5	19.5	
+3	24.0	15.5		40.5	9.5
+4	20.5				10.5
+5	50.0		13.5		13.5
+6	28.0	16.0	17.2	12.0	
+7	16.5			50.5	12.0
+8		18.0	20.5		12.5
+9				44.0	10.5
+10		13.0			

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